Analysis of the genetic and environmental factors affecting grain quality in oats

(*Avena sativa L.*)

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By

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ABSTRACT

Developing high value oat varieties to meet milling industry requirements is constrained by a lack of detailed information on how genetic and environmental differences and interactions, management conditions and levels of N fertilizer impact on grain quality. Focusing on key milling quality characters, i.e. specific weight, kernel content, hullability and thousand grain weight, four winter oat varieties were grown under conventional and organic regimes at six geographical locations in 2012-13 and 2013-14. In addition, grain yields and oil, protein and β-glucan content of the groat was determined, and grain and groat shape parameters were measured using non-destructive methods. Results showed that there was a differential effect of environment on grain chemical and physical parameters and statistically significantly differences for grain and groat area, length and width between varieties and locations (p-value <0.05). There were correlations between grain shape traits and kernel content, hullability and thousand grain weight. None of the varieties displayed a superior performance in all quality traits nor did any one site showed a superior performance over all values for all varieties. Interactions found for chemical quality traits between genotype and environment suggest that niche-matching varieties according to the chemical trait of interest could be conducted. Environments where the varieties were grown displayed variable grain quality results, suggesting that these sites are more suitable to future further investigations on grain quality differences in terms of genotype by environment interactions.

On the basis of previous differential genetic and environmental effects on quality parameters found, in 2013-2014 and 2014-2015, four oat winter varieties were grown under six different levels of nitrogen fertilization. The grain was analysed by non-destructive methods in addition to specific weight, kernel content, hullability, thousand grain weight, and oil, protein and β-glucan content determinations, in order to identify the influence of nitrogen on grain quality parameters. Several non-linear responses with increasing levels of nitrogen on grain quality parameters were found. Specific weight was lower with higher levels of nitrogen. None of the quality parameters positively affected by increasing levels of nitrogen displayed a plateau and thus it was not possible to calculate the optimal amount of nitrogen to apply for a maximal response.

In order to understand the physiological mechanisms involved in panicle development and architecture and how grain quality is affected, a field trial was conducted in summer 2015 and 2016. Three winter oat varieties, Tardis, Mascani and Buffalo were grown and developing grain was sampled at five different growth stages (Zadok decimal growth stage, GS). At each GS and from each variety, a panicle was sampled and divided into individual whorls and within each whorl the primary, secondary and tertiary grain were separated and analysed by non-destructive methods. Measurements of kernel content, thousand grain weight and grain and groat area, length, width and moisture content were taken. The results showed differences between the top and the bottom of the panicle in terms of maturity and also the effect of loss of moisture content during maturation. Each variety showed a unique pattern of development, although some similarities were found between them throughout grain development. Maximal grain width was reached before maximum grain length with both grain shape traits diminishing by final maturity.

Contents

List of abbreviations

AHDB	Agriculture and Horticulture Development Board
ANOVA	Analysis of Variance
cm	Centimetre
ºC	Grades Centigrade (Celsius)
CT	Computerized Tomography
DEFRA	Department for Environment, Food and Rural Affairs
FAOSTAT	Food and Agriculture Organization of the United Nations
g	Gram
G	Genotype (Variety)
GDD	Growing Degree Days
GS	Growth stage
ha	Hectare
HGCA	Home Grown Cereals Authority
hl	Hectoliter
IBERS	Institute of Biological, Environmental and Rural Sciences
KC	Kernel content
kg	Kilogram
l	Liter
m	Metre
Max	Maximum
mg	Milligram
Min	Minimum

ml	Millilitre
mm	Millimetre
n	Number of samples
N	Nitrogen
N/A	Non-Available
ND	Not determined
NIR	Near Infrared Reflectance
N°	Number
NP	Not published
NS	Not significant
NUE	Nitrogen use efficiency
NUpE	Nitrogen uptake efficiency
NUtE	Nitrogen utilization efficiency
PGR	Plant Growth Regulator
RL	Recommended List
rpm	Revolutions per minute
s.e.m.	Standard error of the mean
s	Second
sd	Standard deviation
SNS	Soil nitrogen supply
SMN	Soil Mineral Nitrogen
SpWt	Specific weight
t	Tonne

T	Temperature
TGW	Thousand Grain Weight
trt	Treatment
UK	United Kingdom
USA	United States of America
Y	Year
%	Rate of increase

Chapter one. Introduction

Oats, *Avena sativa*, are a low input temperate cereal grown primarily for its grain. It is an annual plant, and it can be classified as either winter or spring oats. Winter oats are planted in the autumn, over winter in the field and are harvested in the summer. Spring oats are sown in early spring and harvested in late summer.

1.1 Taxonomy

Oats (*Avena sativa* L.), also known as common oat, are part of the family *Poaceae,* also known as *Gramineae*, together with other major grasses of economic importance, e.g. wheat and barley, figure 1.1. The genus *Avena*, with 30 recognized species (Baum, 1977), has a basic chromosome number of 7 with three recognized ploidy levels and four genomes, i.e. diploid (either AA or CC genomes), tetraploid (either AABB or AACC genomes) and hexaploid (AACCDD), being the diploid and the hexaploid found as both, wild and cultivated crops. Comparative karyotype studies and molecular investigations by *in situ* hybridization, and the absence of a DD diploid genome, support the hypothesis that A diploid genomes might be the origin of the AADD genome in the hexaploid oat (Linares, Ferrer & Fominaya, 1998). The genus, with interfertile species, is considered an important gene pool for oat improvement, as in the past has been demonstrated with interspecific transfer of alleles, e.g. disease resistance and oil content (Aung, Thomas & Jones, 1977; Aung, Zwer, Park, Davies, Sidhu, & Dundas, 2010).

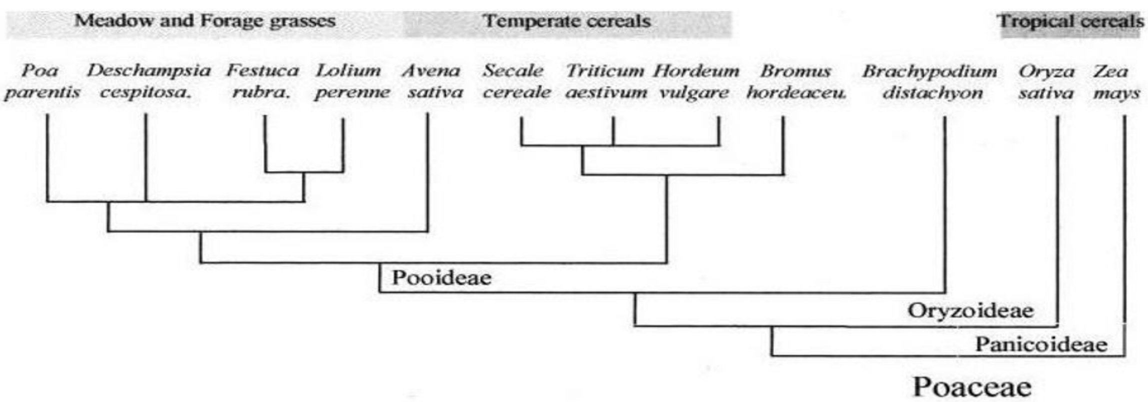


Figure 1.1. *Phylogenetic relationships of species of Poaceae* (Draper, Mur, Jenkins, Ghosh-Biswas, Bablak, Hasterok & Routledge., 2001).

The hexaploid oat, with seven recognized taxa, has a common genome structure, AACCDD. Molecular and genomic studies have shown the close relationship between them, suggesting that the hexaploid cultivated oat, *Avena sativa* L., would have been originated by hybridization and polyploidization combining three diploid sets, AA, CC and DD (Ranhotra & Gelroth, 1995; Linares, Ferrer & Fominaya, 1998; Li, Rossnagel & Scoles, 2000; Loskutov, 2008).

1.2 Oat importance in the market

Oats are grown across the world with 64.2% of the production in Europe and 24.8% in America (1993 to 2014 data, FAOSTATS). Oat production ranks sixth in the world grain production following corn, wheat, barley, sorghum, and millet (Webster & Wood, 2011). The largest producer countries are the Russian Federation (6.2 million tonnes), Canada (3.4 millions tonnes), Finland (1.9 millions tonnes), Poland (1.4 millions tonnes) and Australia (1.3 millions tonnes) average 1993-2014. In Europe, oats are the fifth largest cereal with 14.72 million metric tonnes in 2014. In the United Kingdom, production has increased from 480 thousand tonnes in 1993, to 828 thousand tonnes in 2014, and oats rank fourth in yields per hectare after Ireland, Netherlands and Belgium (FAOSTATS 1993-2013).

Oats are of significant economic importance for human consumption, for livestock feed and increasingly as a source of high value compounds for industrial use. For human consumption, oats are a traditional meal in many countries, as breakfast cereal and porridge. With snowballing interest in eating for health in the developed world coupled with an endemic obesity problem, much attention is being directed towards delivering soluble fibers to the consumer through food. Oats provide more protein, fiber, iron and zinc than other whole grains. They have high nutritive value for both people and animals because of good taste and an activity of stimulating metabolic changes in the body (Bogdanov, 2010).

The recent recognition of oats as healthy food has seen an increase in the use of oats in many products including pasta, bread, biscuits, muffins, cakes, snack food. The value of oats as healthy food is attributed to the presence of β-glucan, and its ability to lower elevated plasma cholesterol and reduce the risk of heart disease. Oat β-glucan can also exert several beneficial gastrointestinal effects, including decreasing the postprandial glucose responses (Wood, 2007), delaying gastric emptying, and increasing satiety (Mak, Virtanen, Malkki, & Virtanen, 2001).

Additionally, preliminary research on minor oat constituents is beginning to establish a link between specific oat components and regulation of allergic responses, asthma, and proliferation of cancer cells (Kasum, Jacobs, Nicodemus, & Folsom, 2002; Ryan, Thondre & Henry, 2011). The beneficial properties of oats are increasingly becoming the focus of researchers with respect to investigating the possibility of developing targeted oat lines to meet the specific needs of industrial end-users using oat as food ingredient, animal feed, whole grain, cosmetics and nutraceuticals.

1.2.1 Challenges

The actual oat market is not only influenced by the necessity for healthy functional foods, There also is a need for high yielding crops to feed an increasing population. As a result of this, the demand for the main cereal crops is increasing and the oat crop has to compete with other cereals and against the increasing concern about the environmental impact of intensive cereal production. Thus, it is necessary to investigate the environmental and genetic factors influencing the key grain quality traits in order to look for improved varieties which meet the end-users requirements and increase grain quality and yield of oat, and at the same time reduce their environmental impact (Marshall, Cowan, Edwards, Griffiths, Howarth, Langdon & White 2013).

High yield in oats, i.e. the productivity of a crop, or more specifically, the number of tonnes of grain produced per hectare grown (Evans & Fischer, 1999), comes from the combination of grain numbers per ear and ears per unit area. The shoot density depends on the quantity of seeds sown, the depth of sowing, tillering at the beginning of the season and tiller survival. The ear density at harvest depends on the number of shoots that produce fertile ears. Balanced crop nutrition of all major and micronutrients is essential to help grow plants that can support this grain (Evans & Fischer, 1999).

The yield formation process can be divided into two interdependent process, development, where the grains are formed and filled, and growth, where the material for forming is provided by photosynthesis (Slafer & Andrade, 1993). The most accepted model is to split yield into its components and it can be viewed as the product of three factors (Evans & Fischer, 1999):

1- Individual (Single) grain weight (SGW)
2- Grain number per panicle
3- Number of panicles per unit area

Grain number per area (GNO)

In other words, multiplying the individual grain weight by the number of grains per unit area is equivalent to the grain yield per unit area. Producing high yields of high quality oats involves interactions among numerous biological factors, management strategies and climatic conditions. Biological factors including disease resistance, straw strength, leaf area, photosynthetic capacity, source-sink relationships and mineral uptake. Good management practices include use of high quality seed at the recommended seed rate, judicious use of fertilizer and pest control (Forsberg & Reeves, 1995).

The two major components of Grain Yield, GNO and SGW are subjected to different conditions and stresses, because they develop during different periods of the growing season. They oscillate in response to resources available. In the most of cases, GNO dominates over SGW, being the determining component for grain yield and depends on crop species and cultivars as well as management growing conditions (Peltonen-Sainio & Rajala, 2007). However, they are interrelated so they can compensate for each other to some extent. The variation that can be founded in GNO is largely attributable to growing conditions which can affect differences in set grains per panicle and numbers of panicle per unit area. Variation in SGW can be attributable to the environmental and management conditions during the grain filling. Thus, the factors affecting yield are determined during different stages and combining improvements over those factors might result in higher yielding oats (Griffiths, 2010).

For the milling industry the objective is to obtain a maximum yield of sound, clean whole oat groat, free from extraneous matter and from them to produce a finished product with an attractive appearance, an agreeable taste, a good digestibility and a good keeping quality, with higher levels of healthy components, e.g. β-glucan. According to the intended use, cereals recommended for cultivation in agriculture should be characterised by a specific colour depending on the end-users, a high content of protein, a good composition of amino acids, a good milling and baking capacity for the foodstuff industry, a high content of digestible protein and a small crude fibre content for animal fodder (Biel, Bobko & Maciorowski, 2009). It is important to know the requirements of the milling industry and of the oat market to focus attention on those traits which are more relevant to end-users. For maximum grain yields, only good quality seed of recommended varieties should be cultivated. Good quality seed will be free of weed seeds, have high germination, be free of cracked, shrivelled and disease seed and be free of seeds of other crops (Forsberg & Reeves, 1995).

One of the gaps that actually exists between lab achievements and field in terms of milling quality is the lack of an accurate method to measure and assess desirable traits of seeds. Nowadays, the most common method used by farmers and the grain trade, is the specific weight, also known as bushel weight or hectoliter weight. This is the weight of grain which fills a specified volume under standard packing conditions, and it depends on the size of the grain, the groat/ grain size ratio is highly correlated with test weight (Doehlert, Jannink & McMullen, 2006). Although the market value of oat grain is largely determined by test weight or bulk density, there is a poor relationship between the specific weight of a variety and its milling quality and as a result, it presents particular difficulties in the selection and recommendation of oat varieties in the field. Quality evaluation by UK millers when purchasing grain generally does not include hullability and kernel content, despite the major implications these characteristics have for mill output and efficiency (White & Watson, 2010).

1.3 Oat agronomy and morphology

1.3.1 Plant and growth stages. Flowering and yield formation

The growth and development of the small grains, wheat, triticale, barley and oat, follow very similar patterns. Oats are an annual plant, completing its development in 6 to 11 months. As a monocotyledon, it has a single cotyledon or seed-leaf.

Grasses produces branches (tillers), at the base of the stem. The leaves differentiate from points on the stems called nodes and are narrow and unstalked almost parallel-sided and parallel-veined. The inflorescences are compound, comprising a series of flowering branches arranged in whorls on which the spikelets are found, which are arranged into a panicle (figure 1.2). Each spikelet has one to several individual flowers, florets. The floret comprises the female part, a superior ovary, and the male parts, the stamens, their number being three or a multiple of three.

The floret is enclosed within two protective bracts or scales, the outer lemma and the inner palea. Following fertilization, the single ovary develops into a caryopsis, comprising an embryo and an endosperm.

The external morphological development of oat plants comprises the achievement of full size of the leaves, tillers, stem and panicle. Individual plants will develop a number of stems depending on growing conditions. The first stem, the main stem, will produce a number of tillers. They arise during the early phase of the life cycle between the emergence of the third leaf and stem elongation. Not all the tillers will survive and in general, older large tillers are more likely to survive than younger smaller tillers. At flowering, most tillers which have reached this stage will bear an inflorescence (White, 1995). The establishment of the best management conditions to the growth and development during this period will influence the numbers of tillers which during flowering time will bear an inflorescence.

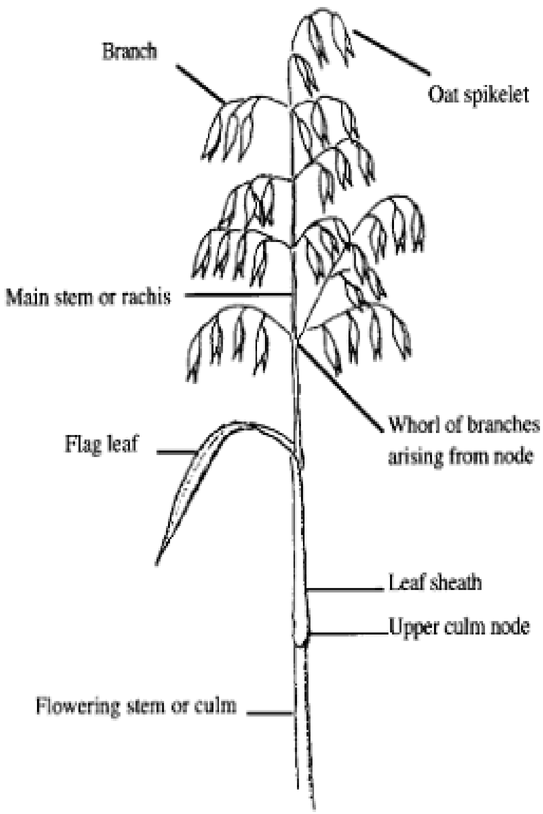
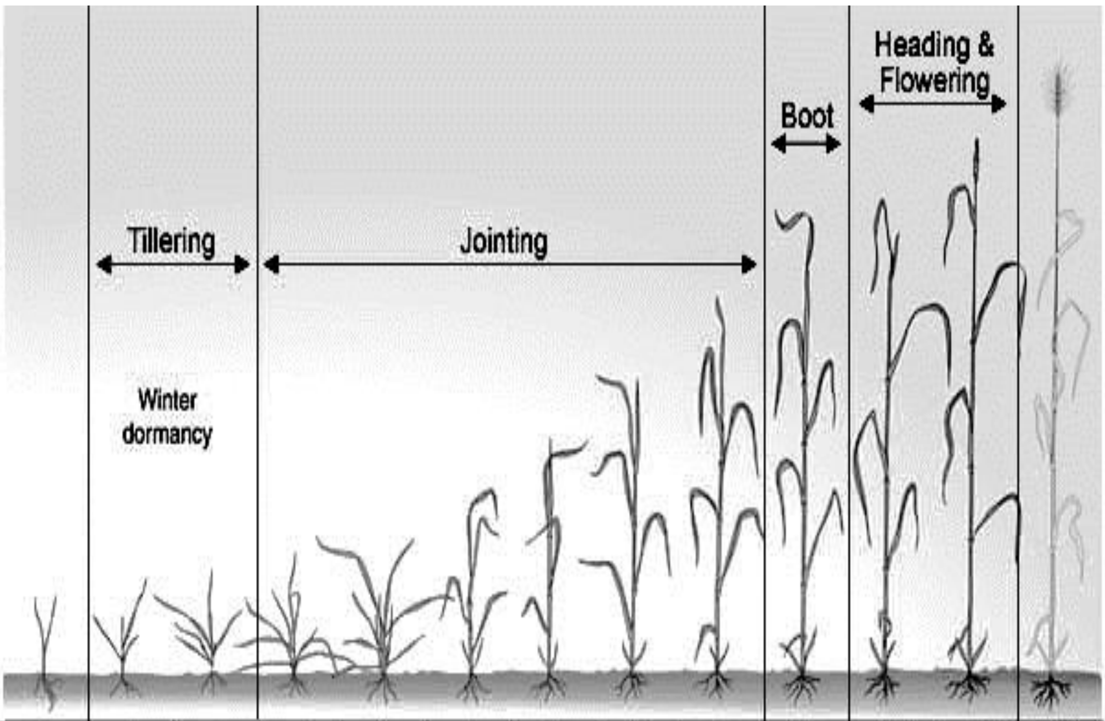


Figure 1.2 *The diagram shows the oat panicle characteristics – flowering stem or culm, upper culm node, leaf sheath, flag leaf, whorl of branches arising from node, main stem or rachis, branch (of rachis), and oat spikelet.(Murray 1980)*

Plant development can be divided into several stages: germination and early seedling growth, tillering and vegetative growth, elongation and heading, flowering, and kernel development. The numbers and states of the external features have been codified into growth stage keys, the Zadoks' decimal code (ZGS) (Zadocks, Chang & Konzak, 1974).

Generally speaking, crop development can be divided into three main phases, from planting to harvest which include all Zadocks stages and are described as foundation, construction and production phases.

The foundation phase, ZGS 00-30 (figure 1.3), starts from sowing and lasts through to the start of the stem extension, including: root growth, leaf production and tillering. Emergence usually occurs 6 to 20 days after sowing, depending on the temperature and moisture. During this time yield-bearing shoots, tillers, and primary roots form as the canopy develops. Each plant has the potential to produce more than 50 tillers. Usually only two to four tillers survive to produce fertile spikes at normal seeding rates and growing conditions. The number of tillers is influenced by plant density, soil moisture and nutrient supply, sowing date, temperature, and cultivar.



Feekes	1	2	3	4	5	6	7	8	9	10	10.1	10.5	11
Zadoks	10	21	26	30	30	31	32	37	39	45	50	60	90

Figure 1.3 *Growth stages in cereals: Illustration of the Feeke's scale. Pl. Path. 3:128-129* (Large, 1954)

The emergence of the primary tillers is synchronous with the emergence of leaves on the main stem of the plant. The initiation of leaves continues until the transition phase of development, the duration of this phase will be influenced by seeding rate and growing environment and by the vernalization and photoperiod requirements of the variety (Brouwer & Flood, 1995). Flowering and grain development is only slightly delayed on later-developing tillers. The components of yield, ear numbers and grain sites/m^2, are set by the end of this stage. The rate of growth will depend on the environment with dull, cool days giving slow growth. In spring oats this phase will be rapid as the days are bright and temperatures increasing (Jackson & Williams, 2006).

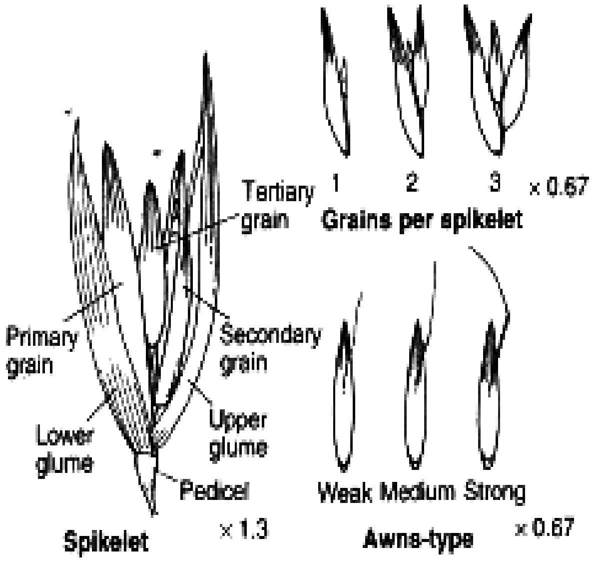


Figure 1.4 A *pedicellate spikelet of the tall fescue panicle inflorescence. It can be appreciated the primary, secondary and tertiary grain inside a leaf like part, i.e. the glume, defining the spikelet (Jarman, Pickett and Eade, 1992).*

The construction phase, ZGS 31-61, starts from the first node being detectable through to flowering. During this stem elongation or jointing period, the stem internodes increase in length and bring the nodes above ground. The uppermost five or six internodes elongate, beginning with the lowest of these. The stem elongation progresses parallel to the appearance of the flowering structure. In oats, in contrast with other cereals, the inflorescence which terminates the stem is in the form of a panicle. Flowering (anthesis, or pollen shed) usually occurs 2 to 4 days after spikes have completely emerged from the boot. This is a critical and very rapid growth period as yield delivering leaves, deep roots, fertile florets and stem reserves form, with a high daily nutrient demand from the soil. By the end of this stage the canopy will be complete (Jackson & Williams, 2006).

The production phase, ZGS 61-92, starts just past flowering, lasting through to grain filling and ripening. Most of the cells inside the grain, are formed during the grain filling, increasing its starch content. The carbohydrate used in this period comes primarily from the photosynthetic output of the flag leaf, at the base of the panicle. During this period the critical yields components, i.e. grains/m^2 and the grain weight will be determined. The health of the flag leaf and its nitrogen status must be maintained as it will contribute up to 70% of the carbohydrate that ends up in the grain (Jackson & Williams, 2006).

1.3.2 The Panicle and Grain development

In oats, the inflorescence which terminates the stem is in the form of a panicle. It consists in a main axis, the rachis, bearing spikelets at their tips. The number and size of spikelets and florets are major determinants of grain yield. The length of the rachis, the number of whorls and the number of primary branches per whorl, control to a large extent the number of spikelets per panicle (Brouwer & Flood, 1995).

The spikelet in oats comprises one, two or three grains; this gives rise to one-kernel, two-kernel and three-kernel spikelets (figure 1.4). The double kernel-spikelet has been found to be the most usual type comprising about 80% of the spikelets (Doehlert, McMullen & Riveland, 2002). The primary kernel is distinctly larger than the secondary kernel and the tertiary kernel (Doehlert et al., 2002; Doehlert et al., 2006). Some commercial interests discourage the production of cultivars with high frequencies of triple spikelets because of potential contribution of the tertiary kernels to the thin fraction that cannot be processed. However, a correlation among genotypes with triple kernel spikelet frequency and percentage of thin kernels has not been found. It appears that secondary kernels from double kernel spikelets contribute as much as tertiary kernels to the thin kernel fraction, and environment rich in nitrogen, that generate more tertiary kernels have been found to also generate larger kernels overall (Doehlert et al., 2006).

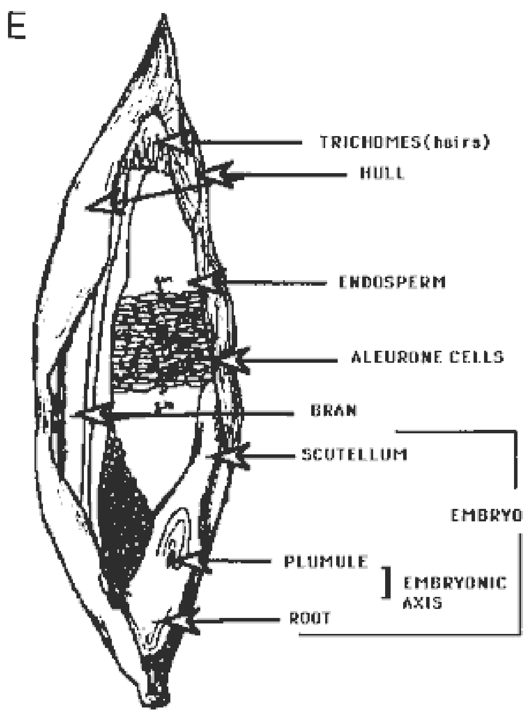


Figure 1.5 *Diagrammatic illustration of the structure of oat kernel (caryopsis fruit) (Haard, 1999).*

The caryopsis, groat or kernel (figure 1.5), in oats is long and elliptical in shape and is covered with fine, silky hairs. It has a rounded dorsal surface with a deep groove on the ventral surface. The ovary wall, seed coat and nucellus comprising several compressed and fused layers of cells together constitute the surface layers, or bran. The endosperm constitutes the greatest proportion of the caryopsis weight, about 80%. The embryo, germ, lies on the dorsal side of the caryopsis, overlaying the lemma. In *Avena sativa* there are no zones of specialized tissue which allow the grains to separate easily from the panicle when ripe (White, 1995).

Once pollinated and fertilised, grain development begins. The grains increase in size and weight as sugars are imported from photosynthesizing parts of the plant and converted into starch which is stored in the cells of the endosperm. Water content inside the grain with respect to its weight, will decrease progressively as starch is accumulated. This starch will be laid down in the grain as long as the plant continues to photosynthesize. During this period, the oat crop is susceptible to lodging. The properties of the material comprising the internode walls contribute to the resistance to lodging (Marshall & Sorrells, 1992).

The last of the stages of the plant development is ripening. Before fertilization, senescence of the plant begins, as individual leaves only function for a limited period. The panicle gradually loses its ability to photosynthesize. The whole plant dries out and the grain becomes harder as its water content decreases (White, 1995).

One of the most important aspects in grain development is to establish the moment of maximum growth in order to harvest the oat when it is more suitable in terms of high kernel content, specific weight and moisture conditions. Nowadays, oats are often harvested when grain is in the hard dough stage and straw is slightly green. In some regions, harvest date is governed by the weather, and in order to avoid possible diseases, weeds and insects. If oats are left to dry down in the field they can weather. The surface of the kernel might be attacked by a fungus and discolour or turn black. This is undesirable as dark kernels are unacceptable for milling. To get the best quality, oats should be combined as soon as they are ripe but without compromising high standards in grain quality parameters.

However, the differences of growth between the top and the bottom spikelets of the panicle and among the grain found inside the spikelet (Griffiths, 2010), can result in a mix of completely and not completely mature grain which can reduce the specific weight and the kernel content of the cultivated oat.

1.3.3 Naked and Husked Oats

The presence of the husk in oats after harvest allows us to differentiate between naked and husk oats. Naked oats is a variant of *Avena sativa* which, when it is harvested, are caryopses without their enveloping lemmas and paleas. This variant is a feed suitable for monogastric animals, with higher protein and fat contents and lower fibre content than husked oats, but with lower yields. Removal of the fibrous husk has an important effect on the metabolizable energy content and increases the proportion of other nutrients relative to other cereals (Valentine, 1995).

In husked oats, the husk consists of a thick fibrous lemma and smaller thinner palea which surround and protect the caryopsis. The thick fibrous husk can lower the energy value of the grain for feeding to livestock and its proportion to kernel can vary considerably between environments and varieties, but at the same time can play a protective role for the caryopsis from damage which could lead to rancidity or reduction in germination (Valentine, 1995). In naked oats, the lemma is non-lignified and appears thin and papery, like the glumes from which naked grain threshes free. The spikelets of naked oats, are typically multiflorous, in contrast to the compact spikelets of husked oats which usually contain only two or three functional florets (Jenkins & Hanson, 1976). Husked oats usually have two or three fertile florets per spikelet, whereas naked oats have a multiflorous spikelet. The terminal fertile florets in naked oats have progressively smaller grains, and the spikelets are soft, non-lignified. Nakedness is affected by modifying genes and environmental factors. Some genotypes, “mosaics”, have a high proportion of husked grain, usually in the basal whorls of the panicle and in the terminal parts of the inflorescence (Valentine, 1995). Nakedness is therefore incompletely dominant and appears to have pleiotropic effects, with a major “switch” gene, N, which is modified in its expression by three other loci (Jenkins & Hanson, 1976).

The husk is an important structure considering that it is necessary to remove it in order to get the kernel or groat by a method named the dehulling process. There are two major mechanical methods, compressed-air dehulling and impact dehulling, both with very different results depending on dehulling conditions, which represents a compromise between unfavourable extremes. Both methods present better results if a secondary pass is done, resulting in higher dehulling efficiency, but increasing groat breakage as well (Doehlert, 2001). In a compressed air dehuller the grain is subjected to compressed air to achieve a separation of kernel and husk. In this process, the husks are suctioned off with a fan and collected separately. Impact dehulling involves feeding oat grain into the centre of a spinning rotor that expels the grain against the walls of the dehuller. The force of the impact breaks the hull from the groat. Dehulling efficiency increases with rotor speed but groat breakage does as well, which increases the presence of screenings and reduces the milling quality. Maximal unbroken groat yield represents a balance between dehulling efficiency and groat breakage (Doehlert, Wiesenborn, McMullen, Ohm, & Riveland, 2009).This process is influenced by the presence and the different size of the primary, secondary and tertiary grains, which determine the required time and speed, necessary to get the caryopsis (kernel, groat) from the grain (White & Watson, 2010).

Many factors affect the hullability in oats. Oat cleaning and processing is based on physical characteristics such as size, shape and density of kernels. Although kernels are sorted according to size to improve dehulling efficiency, the final milling yield is also influenced by the combination of kernel feed rate and dehuller speed (Symons & Fulcher, 1988).

Moisture content has an important role in affecting hullability. During the ripening of the grain, the moisture content decreases, allowing the kernel/groat/caryopsis, to separate from the lemma and palea, i.e. the husk, increasing the hullability and in consequence, the milling quality of the cultivated oat (Doehlert, 2001). Regarding agronomic factors, hullability has been found to improve in crops grown at higher rates of nitrogen, but is poorer at higher seed rates and with the application of plant growth regulator. Management for quality should be focused on choosing varieties that meet the quality criteria used in the commercial trading of grain and that are important in determining milling quality (Browne, White & Burke, 2004).

On the other hand, analysis of panicle architecture reveals significant environmental effects, genotype effects and genotype by environment interaction, among naked and hulled genotypes for the number of kernels per spikelet, kernels per panicle, grain mass per panicle and mean mass per kernel (Doehlert et al., 2006). Kernel size has been found to decrease with increased order within naked oat spikelets, with more uniformity in kernel size than hulled kernels. Thus, much of the difference in kernel size distributions between naked and hulled oat can be attributed to the presence of the hull, which can result in larger kernel size, being the naked oat kernel size more uniform than hulled oat (Doehlert et al., 2006). Despite these significant effects, the genetic mechanism and the effect of the geneotype by environment interaction that directly affect this variation remain unclear.

1.4 Milling quality

Countries tend to establish their own set of grading standards for oat quality. The factors that determine milling-oat quality are: genetic, environmental, nutritional, storage-management, and handling. Each oat variety has a specific ratio (width/length), kernel weight and presence or absence of awns, all functions of the genetic background. All these factors affect the milling quality (Girardet, Webster & Wood, 2011). In the United Kingdom, the minimum specific weight to accept a crop in the market is 50 kg/hl. In general, for the milling market, oats should have a high specific weight, high kernel content, good hulabillity and low screenings. To meet this requirements it also has to have a high yield and stiff straw, and good resistance to diseases.

1.4.1 Specific Weight

Market value of oat grain is largely determined by specific (also known as test, or bushel) weight, and yet little is known of the physical basis for specific weight in oats. The specific weight is the weight of grain which fills a specified volume under standard packing conditions.

Although the specific weight is regarded as a good measure for grain quality, it has shown a poor suitability to predict potential milling yield (as a function of kernel uniformity, hull content and percentage of thin kernels in the sample), particularly potential extract yield (Burke, Browne, White, & Park, 2001; Girardet et al., 2011). Despite it being an easy measurement to perform, it has some limitations in making yield predictions between varieties and assessing directly important characteristics related to milling quality such as kernel content and hullability. Some varieties can have an excellent yield and kernel content values but low specific weight and because of this they are rejected for the market, the milling industry and the farmers. These variable results imply that there are variety and environmental factors that affect specific weight without influencing milling yield (Pushman & Bingham, 2017).

Studies of the relationship between specific weight and other milling quality traits have shown variable results. Positive correlations with kernel content, have been previously reported (Doehlert, 2001; Peterson, Wesenberg, Burrup & Erickson, 2005; Achleitner, Tinker, Zechner & Buerstmayr, 2008; Genotypes, Mut, Doğanay, Kose & Akay, 2016), but for a single cultivar.Other researchers, Browne, White, & Burke (2002), did not find correlation between a high specific weight and kernel content when comparing varieties. The differences found regarding the specific weight and kernel content between varieties are most likely related to hull and groat size characteristics, i.e. length and width. This is might be explained by the presence of the hull contributing to mask the real size of the kernel due to the empty space inside the grain between the hull and the kernel that can reach 16% of the total (Browne et al., 2002). This may result in lower specific weight. Thus, thin, tight fitting hulls appear to contribute to high specific weight and would be reflected by high groat to oat grain size ratio. It can be concluded that the groat/grain size ratio is a fundamental aspect for the physical basis of the methods to specific weight (Doehlert et al., 2006). Depending on the variety, the hulls are tightly wrapped around the groat, while in others, the hulls are more loosely associated with the groat. This could create differences in packing characteristics (Girardet et al., 2011), and therefore in the specific weight of the varieties.

This space between the husk and the kernel depends on genetic, environmental and agronomic factors during the grain filling period and may vary between years as well. Other factors affecting the specific weight are rust, drought, lodging, late sowing, high seed rates, which can reduce the dry matter accumulation throughout grain filling, and therefore decreasing resources to fill the grain. These factors might lead to smaller groats by increasing the empty space inside the grain and/or lighter grain, affecting specific weight values. Being variety (White, McGarel & Ruddle, 2003), environment and agronomic management the main factors influencing specific weight it will be the focus of study in this project.

1.4.2 Thousand Grain Weight

The thousand grain/kernel (TGW) weight is a measure of seed size. It is the weight in grams of a thousand seeds. Kernel weight is an indicator of kernel size and density. It is determined by counting and weighing 100 or 1000 kernels and it is expressed as grams per thousand kernels or thousand kernel weight. Alternatively, it can be expressed on a single-kernel basis in milligrams.

TGW is an important parameter in the determination of the most appropriate seed rate to sow to get a maximum yield. By using the TGW, a producer can account for seed size variations when calculating seeding rates, calibrating seed drills and for setting up the combine for harvesting to minimise shattering and combine losses. The optimal plant density results from the establishment of a certain number of plants per square meter. The tillering capacity of each variety can also affect the number of panicles per plant. It may be argued that high seed rates and therefore high plant populations will increase the number of panicles and grains per square meter; however, due to the responses of yield formation processes to agronomic factors this may not be necessary true. The competition that is derived from a higher number of grains per panicle for the photosynthate can result in a higher number of aborted grains and therefore reduce seed number, increase the presence of empty husks and affect the quality of the grain (Browne, White & Burke, 2006).

The physical bases of individual grain weight are determined by the number of grains per panicle, size, shape and composition of the kernel and from an agronomic point of view by the duration of the grain filling period. The final grain dry weight within and between varieties can be quantified in terms of differences in the duration of the lag and linear phases of the development. However, for a specific variety, this parameter remains relatively stable due to a more flexible grain number establishment and the mechanism of aborting grains when the assimilates available are low or by filling the tertiary grains when the assimilates are high (Browne et al., 2006; Peltonen-Sainio, Kangas, Salo & Jauhiainen, 2007). This phenomenon suggests that the variability within and between varieties depends on genetic factors, and this allows for the selection and breeding of varieties with fewer grains per spikelet and more spikelets per panicle with a more uniform grain weight.

1.4.3 Kernel Content /Groat percentage

The weight of the kernel relative to the weight of the oat grain is referred to as the kernel content. It is the principal factor affecting milling yield, and it may influence the actual method to determine the market value of an oat variety, the specific weight (bushel weight or hectolitre weight). It is defined as the amount of hull-less kernels obtained after dehulling, expressed as percentage of weight of the sample.

The kernel content, also known as groat percentage, represents an important quality characteristic of oat affected by the mechanical factors that arise from the oat dehulling process and the physical characteristics of the oat grain. Mechanical factors consist in the strength and duration of mechanical stress required to separate the hull from the groat and the strength of the aspiration required to remove free hulls from the groats. Insufficient mechanical stress can result in ineffective dehulling, but excessive stress may increase the groat breakage. Additionally, excessive aspiration remove groats as well as hulls, but insufficient aspiration leaves excessive hulls with groats, which may result in the devaluation of the grain since the millers are interested in cleaned samples.

Oat size uniformity appear to be highly correlated with kernel content. The negative correlation between hulls remaining after dehulling and kernel content, and the positive correlation between groat breakage and kernel content suggest that heavier hulls are more difficult to remove whilst thin hulls provide less protection to the groat during dehulling (Burke et al. 2001). Thus, there must be a compromise between increasing the kernel content and the hullability and decreasing groat breakage.

Compressed-air dehullers provide one possible option for rapid evaluation of kernel content and could possibly be of value to determine the quality in husked oats, in terms of remaining hulls and groat breakage.

1.4.4 Grain Size

The grain dimensions that define the size of the kernel are area, length, width and depth. They are influenced by both genotype and environment and by its interaction. In general terms, millers, in order to get higher yield of white flour or soluble extract and large traditional flakes, prefer large and uniform round grains (Doehlert et al., 2006). At the same time, the kernel size and its uniformity affect the efficiency of the dehulling process.

Oat grain size is determined by the plants genetics i.e. variety, and the length of the grain filling period and the environmental conditions during grain filling. As soon as pollination occurs the embryo and endosperm begin to develop with the plant redirecting photosynthates and the previously stored starch and protein (in leaves and stems) to these developing grains. The longer this period of grain fill is the larger oat grain size is likely to be.

Additionally, the differences in the structure of the inflorescence in oats and of its constituent spikelets and grains, have implications for the distribution of photosynthate during grain filling and this may affect the grain size and thus the quality (Browne et al., 2006). Some studies (Symons & Fulcher, 1988) have suggested that the grain population structure could be a potential quality parameter for a variety due to the variation in grain size between the two main subpopulations within the grain lot and their specific and different contribution to quality traits.

The primary grain is larger and has lower kernel content and poorer hullability than secondary grains. Tertiary grains have lower mean grain weights and higher kernel contents than secondary grains (Doehlert et al., 2006; White & Watson, 2010). These differences in grain within a panicle are the cause of the grain size variations in samples of oat and affect parameters like the specific weight and the hullability, which determine the quality in the milling market (Doehlert, Jannink & McMullen, 2008). Analysis of histograms of length, width and area distributions of the size fractions found suggest that the oat size populations are composed of at least two distinct subpopulations (Doehlert, McMullen, Jannink, Panigrahi & Riveland, 2004). This bimodal distribution can be attibruted to the architecture of the oat spikelet (Doehlert et al., 2008), where primary kernels from the two-kernels spikelets make up the larger kernel subpopulation and the secondary kernels make up the smaller kernel subpopulation. Aditionally, this distribution creates different optimal conditions and therefore inefficiency in processing oat, due to the necessity of segregating the sample between the primary and the secondary grain subsamples for the dehulling process (Symons & Fulcher, 1988).

Little is known about how development in oat panicles and grains is affected by genetic and agro-ecological factors. Study of the variation in the shape and size of the kernel, i.e. the area, the length and the width, will help us to understand the different stages of development of the panicle and of the primary, secondary and tertiary grain inside the spikelet. The mechanism by which the grain dimensions are established and the influence of the environment and how far they are under genetic control, may enable us to characterize the process of grain development and the specific requirements of every stage. This can result in a better knowledge of the best conditions and selection of the best varieties for a high yield on the basis of grain size, reducing the variability of the weight between subpopulations within a variety and increasing specific weight.

1.4.5 Grain Composition

The chemical composition of the groat or caryopsis also has an impact on aspects of oat nutritional quality. Oats contain more soluble fibre than any other grain, they are high in the fatty essential fatty acid, linoleic acid (Youngs, 1986; Zhou, Robards, Glennie-Holmes & Helliwell, 1999) and constitute a healthy source of proteins, vitamins and minerals, with high levels of antioxidants, α-tocotrienol and α-tocopherol, and avenanthramides, which are unique to oats. The functional quality of oats determines the process after harvest. How grain is processed and the response to that process by the grain may affect the acceptance of the product by the end-user and the consumers (Miller & Fulcher, 2011).

The chemical composition of the hull may also have an important role in the hullability of the grain, which affects the efficiency and economics of the milling process.The hull consists of the remains of modified leaves (palea and lemma), composed of empty cells with lignified secondary walls. Two major constituents of the hull are cellulose and hemicellulose and lesser amounts of lignin and phenolic compounds. The concentration of lignin in the hull is directly related with its digestibility and play an important role in the quality of oats as forage crop (Miller & Fulcher, 2011). There is evidence of the variability in the content of lignin between oat varieties, making them more digestible and suitable for feeding (Crosbie, Tarr, Portmann & Rowe, 1985). Oats with low lignin husk are good candidates to breed for new varieties with low lignin content, in order to improve the hullability of the current varieties and make them more suitable as a feeding crop.

The groat comprises the bran and the endosperm (figure 1.5). These layers contain protein bodies, lipids, soluble fiber, and phenolics compounds. Oats contain more soluble fiber than any other grain, which results in a slower digestion and an extended sensation of fullness. The recent reports of the beneficial physiological effects of the soluble fiber, the β-glucan, have increased the interest in oats as healthy food source. Epidemiologic and clinical studies suggest that dietary factors in addition to the intake of fat and cholesterol influence the degree of risk of coronary heart disease. Human experiments have shown that the oat fibre tends to lower plasma total and LDL cholesterol. Additionally, the low glycemic index of oats is beneficial for people with diabetes and might lower plasma lipids, as well as increasing the transport of bile acids (Maket al., 2001; Xu, 2012; Andersson, Immerstrand, Sward, Bergenstahl, Lindholm, Ste & Hellstrand, 2017).

β-glucan, i.e. (1→3)(1→4)-β-D-glucan, has been proven to help lower cholesterol. It is the main component of the soluble non-starch polysaccharide fraction of oats primarily located in the outlayer of the endosperm, i.e. the bran. It is a viscous polysaccharide composed of a mixed-linkages which make it soluble and flexible. The β-glucan content varies between and within varieties, ranging from 2 to 8 gramms per 100 gramms in oat groats. These differences are due to the size of endosperm cells, the thickness of the cell walls throughout the groat. The distribution and molecular weight of β-glucan vary widely among different cultivar varieties (Sikora, Tosh, Brummer & Olsson, 2013). To efficiently breed oat cultivars higher in this beneficial constituent, the influence of genotype and environment must be determined.

Lipids, proteins and starch are the main storage products in oat grain and these are important also in determining grain quality. The oil content (synonymous with lipid content) in the kernels of different oat cultivars varies from 3 to 12% of the dry weight, while the protein content ranges from 16 to 20% and the starch from 45 to 60% of dry weight. The differences observed are due to the different activities of the enzymes in the different kernel tissues (Banaś, Dahlqvist, Dêbski, Gummeson & Stymne, 2000). The bran and the endosperm contain the higher fractions of the most important essential lipids that we can find being linoleic, palmitic, oleic and in minor amount stearic and linoleic (table 1). Lipids are of importance due to its impact on nutritional quality and in the flavor and off flavour attributes of oats (Zhou et al., 1999).

Table 1.1. *Average chemical composition for oats (g/100 g)(Welch, 1975; Webster, 2011).*

	Oats
Moisture	13.1
Proteins	10.8
Available carbohydrates	56.2
Fiber	9.8
Minerals	2.9
Vitamin B_1	6.7
Vitamin B_2	1.7
Nicotinamide	24.0
Panthothenic acid	7.1
Vitamin B_6	9.6
Folic acid	0.3
Total tocopherols	18.0
Lipids	7.2
Palmitic (C16:0)	18
Stearic (C18:0)	2
Oleic (C18:1)	18
Linoleic (C18:2)	56

Although the total oil range in average is between 4-6% in oat grains (including the husk), there are also wide variations between varieties, some of which contain only 2% whilst others can reach 8% oil content. This range is influenced by genetic and environmental factors. Low growth temperature increases the overall lipid synthesis, particularly oleic and linoleic acids and decreases the concentration of palmitic and stearic (Canvin, 1965; Saastamoinen, Kumpulainen and Nummela, 1989; Banaś *et al.*, 2000). Negative correlations have been found between oil and protein content although this interaction appear to be not consistent and due to genetic and environmental factors (Welch & Leggett, 1997).

Oat protein content varies substantially within cultivars from the same region (Welch & Yong, 1980) reflecting the differences in the availability of soil nitrogen. The application of fertilizer to the soil has been proved to increase the protein content (%) in the grain. Although, it has often been found negative correlation between grain yield and protein content (Simmonds, 1995), other results show any significant decreases in grain or groat protein with increasing yields. Thus, it can be argued that there is a scope for increasing oat protein content without incurring a yield reduction (Welch & Leggett, 1997).

The high nutritional protein value of oats has been confirmed by the analysis of the amino acid composition. Comparison with other cereal species and grasses shows that there are higher levels of cysteine, histidine, isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan, tyrosine and valine, all of them essential amino acids (Welch & Yong, 1980). Although several studies (Pomeranz, Robbins & Briggle, 1971; Peterson & Smith, 1976), show that there is a range between the amino acid composition, i.e. protein quality, and genotypes and environmental factors, the low correlation found in these studies suggests that there is a scope for selection of varieties with high protein content but without significant loss of protein quality (Pomeranz et al., 1971).

Tocols and avenanthramides are secondary metabolite compounds found in oat grain which are of interest for their possible healthful effects in diet (Ryan et al., 2011). They are considered as antioxidants and its variation due to both genetic and environmental conditions of grain production has been documented (Emmons & Peterson, 2001; Fogelfors & Peterson, 2004). These traits should be a focus for the breeders in order to get varieties with high levels of these compounds and therefore increase the nutritional value of future varieties.

1.4.6 Effect of genotype and environment on milling quality traits

Genotype and environment are major determinants of plant phenotype. Economically important quantitative traits include agronomic characteristics and grain composition: specific weight, kernel content, thousand grain weight, hullability, grain size and grain composition, i.e. oil, protein and β-glucan content. Several different investigations studying the effect and the interaction of genotype and environment have shown significant differences within and between varieties for all traits (Brunner & Freed, 1994; Groh, Kianian, Phillips, Rines, Stuthman,Wesenberg, Fulcher & Stuthman, 2001; Peterson *et al.*, 2005), through environments and harvest seasons. However, the magnitude of the effect of both genotype and environment, and their interactions, on all quality traits was variable (Peterson, 1991; Brunner &Freed, 1994; Doehlert, 2001, 2002).

1.4.7 Fertilization and management conditions

Increasing the competitiveness of oats with other cereals, requires an optimum rate of Nitrogen fertilization, minimising environmental impact and maximizing milling industry and farmer's benefits. Given the fact that crop production is the single largest cause of the anthropogenic alteration of the amount of nitrogen that enters the element´s biosphere cycle, nitrogen management conditions should consider soil nitrogen supply, previous crop and inherent soil fertility (Brunava, Vilmane & Zute, 2015; Smil, 1999), to avoid undesirable losses.

Oats are described as a low input cereal (Dawson, Huggins & Jones, 2008; Kindred, Verhoeven, Weightman, Swanston, Agu, Brosnan & Sylvester-Bradley, 2008), needing lower nitrogen fertilizer compared with other cereals. For example the recommendation in the United Kingdom is a maximum of 160 kg ha^{-1} nitrogen for winter oats compared to 250 kg ha^{-1} for wheat (HGCA, 2009). The excessive application of fertilizers might cause lodging of the crop and a lower specific weight, grain quality and yield.

1.5 Introduction to this project

Grain quality of oat is measured in various ways. For the milling industry, quality is measured by milling yield, or the weight of grain from which 100 kg of millable groats are obtained (Groh *et al.*, 2001). Since only larger groats are millable, the ratio of primary to secondary and tertiary kernels is important to millers. For animal feed, grain quality is measured by kernel content or groat to hull ratio because the groat has a greater digestibility and nutritional value than do the hulls. Thus, grain size and shape and its relation to kernel content, seed weight and proportion or ratio of primary to secondary, are important parameters with potential to relate to milling quality parameters. The chemical composition of grain, such as oil content, fatty acid composition or beta-glucan content can be important quality factors for specialty markets for oat.

Therefore, it is necessary to develop accurate methods testing kernel content, specific weight and other quality parameters. Non-destructive Image Analysis (Near Infrared Spectroscopy and digital seed analyser) has the potential to provide a high throughput and rapid alternative method for assessing grain quality and will be evaluated during this project.

The mechanisms by which the oat crop produces stable quality characters alongside large-scale variation in yield in response to agronomic and environmental factors are poorly understood. Thus, this project will focus on three lines of investigation interrelated, i.e. oats development, panicle architecture and relation with yield and milling quality parameters.

Experimental chapter three will investigate effects and interaction of genotype and environment on grain dimensions and panicle architecture. Grain and groat milling quality parameters, and grain dimensions, i.e. grain length, grain width, grain length-to-width ratio, grain area, and grain ratio, have been found to be positively correlated with grain weight (Marshall *et al.*, 2013). Their relationship to both kernel content and specific weight from several different populations grown in different sites and under different agronomic conditions will be analysed.

Experimental chapter four will focus on analysing the effect and interactions of different levels of nitrogen fertilizer on yield, milling quality parameters and grain and groat size. Nitrogen fertilizer have been proved to be of importance affecting yield crop, with variable results, its agronomic implications, including cost/effective production and as a factor of environmental impact (Chalmers, Dyer and Sylvester-Bradley, 1998). The search for an optimum level of nitrogen that increase yield and milling quality parameters will be the main hypothesis to test.

Experimental chapter five will investigate oats panicle development. Focusing on grain development, the analysis of the differences along the panicle and between varieties, and the relation with kernel content, and thousand grain weight, will help to have a new insight of panicle structure. This will lead to a better understanding of the mechanisms underlying the relation between milling quality parameters and grain dimensions, i.e. grain size and shape.

Chapter two. Material and Methods

2.1 Cultivars under study

Five winter husked oat varieties from the Aberystwyth breeding programme (table 2.1), were used in this research. The varieties were chosen either due their importance for U.K. agriculture during the period of study or because they are parental lines for genetic mapping studies. All varieties have been on the U.K. recommended list but not all at the same time. Each chapter within this thesis has a specific experimental design involving a subset of these varieties and this will be described in each relevant chapter.

Table 2.1. *Data from AHDB Recommended List trials for the five winter oat varieties used in this thesis. Values are the means for the harvest years 2007/08, 2008/09 and 2009/10 except for Buffalo for which the data is the mean from the last three years it was in recommended list trials.(AHDB Cereals & Oilseed, no date). Ripening is determined as days ± Gerald -ve=earlier; Screenings is % through 2.0 mm sieve; Lodging and disease resistance are scored on a 1-9 scale where high figures indicate that a variety shows the character to a high degree.*

***Non available*

		Quality			Agronomic Features			Disease Resistance	
Variety	*Grain Yield*	*Kernel Content*	*Specific Weight*	*Screenings*	*Lodging resistance*	*Height cm*	*Ripening days*	*Mildew*	*Crown Rust*
Balado	9.34	73.6	50.4	3.3	8	86	+1	4	3
Gerald	8.56	72.8	52.9	3.1	6	110	0	3	5
Mascani	8.67	78.2	54.2	1.7	6	109	-1	6	8
Tardis	8.76	72.9	49.5	*na***	7	105	-2	8	*na***
Buffalo	8.26	70.3	50.7	4.0	8	97	0	2	6

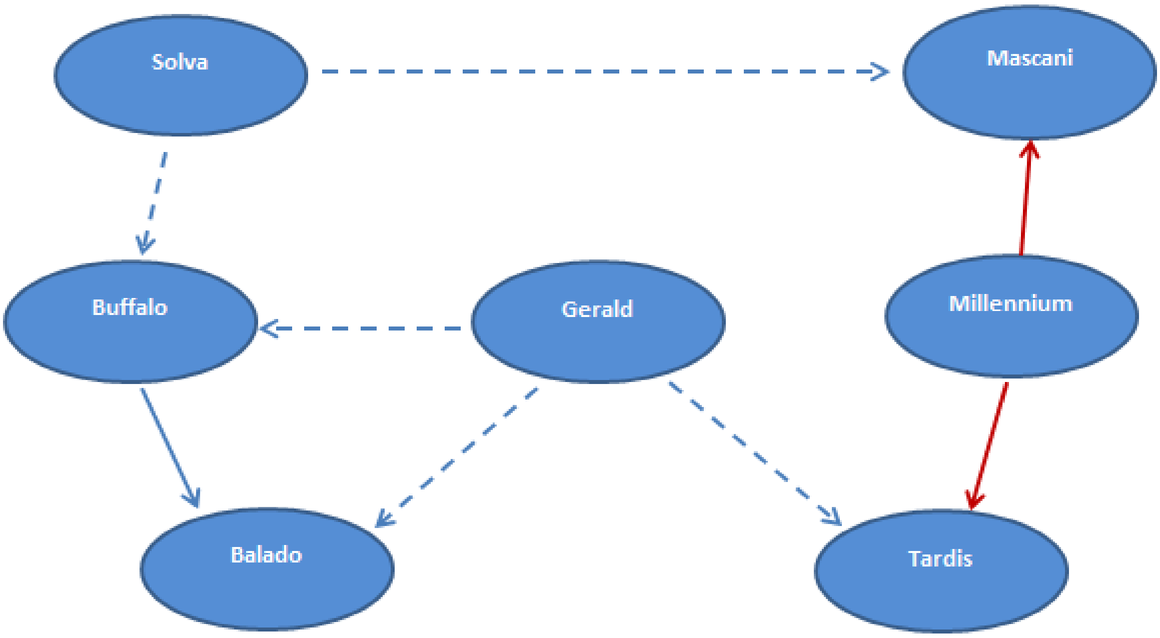


Figure 2.1. *Genealogical/Breeding relationships between varieties used in this research. Red solid line represents direct parents in the breeding process. Blue and blue dotted lines represent the presence of the variety on the pedigree (Howarth, C. personal communication).*

The varieties used in this research are related by pedigree as shown in figure 2.1. Gerald was the oldest variety used and is a grandparent of Buffalo and a great-parent of Tardis. One of the parents of Gerald is the variety Solva, a popular variety until 1995, and is found somewhere in the pedigree of all varieties used. Buffalo and Balado are both dwarf varieties which were bred-by the backcrossing of the dwarfing gene *Dw6* from the spring oat Canadian line OT207 into Solva and then further crossed with UK varieties (Milach, Rines & Phillips, 1997). The variety Millennium, a large grained variety which was on the recommended list from 2000 until 2006, is a parent of both Mascani and Tardis. Tardis incorporates Pc-54 which has provided a highly effective source of adult plant resistance to mildew, *Blumeria graminis* f. sp. *avenae, crown rust, Puccinia coronata*, and oat mosaic virus, although this resistance is affected by environmental conditions (Clifford, 1995).

Currently, Mascani is the most popular winter oat grown in the U.K., with over 68% of winter oat certified seed available in 2017 (Senova personal communication). Mascani, Gerald and Balado are either current or former control varieties for the AHDB Recommended list trials and were used as controls in all the multi-location field trials of

IBERS advanced breeding lines during the period of experimentation covered in this thesis, hence their inclusion in this study. Tardis and Buffalo, although now outclassed as varieties are the parents of a mapping population used in this thesis. Varieties were selected within each experiment according to their suitability to the research question and availability in the experimental framework. Sowing and harvesting times, locations and management conditions, are specified in each individual chapter.

2.2 Methods. Measurements and quality parameters

2.2.1 Weather conditions

Meteorological data was obtained either by the use of on-site weather stations or using locally located publicly available Met office sites. Weather conditions measured included temperature, minimum and maximum (^{o}C), relative humidity (%) and rainfall (mm) on a daily basis. These parameters were used to calculate, where possible, the growing degree days (GDD).

Growing degree days *(GDD)* is a weather-based indicator for assessing crop development, used by crop producers. It is a measurement of heat accumulation used to predict plant development and the date that a crop reaches maturity.

Plant development depends on temperature and requires a specific amount of heat to develop from one point in their lifecycle to another, such as from seeding to the harvest stage. Temperature is a key factor for the timing of biological processes, and hence the growth and development of plants. When there are no extreme conditions such as drought or disease, plants grow in a cumulative stepwise manner which is strongly influenced by the ambient temperature. Many developmental events of plants and insects depend on the accumulation of specific quantities of heat, thus, it is possible to predict when these events should occur during the growing season regardless of differences in temperatures from year to year. GDD units can be used to assess the suitability of a region for production of a crop, estimate the growth stages of crops, weeds or even life stages of insects, predict maturity and cutting dates of forage crops. Daily growing degree day values are added together from the beginning of the season, providing an indication of the energy available for the plant growth. GDD totals are used to compare progression of a growing season to the long-term average.

Growing degrees (*GD*) is defined as the mean daily temperature (average of daily maximum and minimum temperatures) above a certain threshold base temperature accumulated daily in time. The base temperature varies between crops and the value is derived from the growth habits of each specific crop. It is that temperature below which there is not plant growth. In oats, similar to barley, rye and wheat, it is 4.4 ^{o}C or 40 ^{o}F (Miller, Lanier & Brandt, 1997).

GDD were calculated each day as described in equation (1) in which the maximum temperature (T_{max}) plus the minimum temperature (T_{min}) is divided by 2 (in other words the mean temperature), minus the base temperature (T_{base}). GDD are accumulated by adding each day´s GDD contribution as the season progresses. If the average temperature is below the base temperature, the growing degree day value for that day is zero.

$$GDD = (T_{max} + T_{min}) / 2 - T_{base} \qquad (1)$$

If the T mean ((T max+Tmin)/2) term, is less than T base, then GDD is zero.

GDD are typically calculated from the time of sowing.

2.2.2 Yield and grain quality

Grain used for in this research and described in subsequent chapters was harvested using a small plot combine and harvested yields corrected to 15% moisture content. Harvested grain was cleaned through a 3.5 mm and 2 mm sieve for subsequent analysis of grain quality to get rid of straw, double grains, undesirable particles, etc. but cleaning losses were not determined.

2.2.3 Specific Weight

Specific weight (kg/hl), also known as hectolitre weight or test weight is defined as the weight of grain which fills a specified volume under standard packing conditions. Cheap and easy to perform, and with little technical training required, it is the actual method used by the grain trade to determine the market value of oats as it affects the weight of grain contained in each lorry load transported. Previous studies however, have shown that it is not related to key milling quality parameters such as kernel content or hullability (Burke et al., 2001; Manley, Engelbrecht, Williams & Kidd 2009).

Specific weight was measured using a chondrometer (C288) on three replicate samples (approximately 500 ml) per field. Chondrometers are cylindrical devices containing a column in which grains are isolated from the cylinder of known volume underneath by means of a level blade or metal bar (Manley *et al.*, 2009). The blade separates a precise volume of grain (below the blade) from excess grains above the blade (ISO 1986). The upper part, the forerunner, was filled with the sample to the top. Then a little trap door allows to the sample to drop into the bottom container. With a cut off slide, the excess of sample was removed from the rest. This known volume of grain was weighted and the mass converted to kg hl^{-1}.

2.2.4 Thousand Grain Weight. Kernel Content and Hullability determination

From each location and variety, thousand grain weight was also calculated (TGW). A 30 g sample, from each of the three replicates per variety from each location and harvest season, was counted out by a seed counter (Data technologies model number data count S-25) and weighted in a precision scale. The data obtained were used to calculate TGW following the equation below.

$$Thousand\ Grain\ Weight = \left(\frac{Average\ weigth\ of\ a\ hundred\ grains}{100}\right) x\ 1000 \quad (2)$$

Kernel content is the mass of groat or kernel relative to the mass of the grain. It represents the highest priority in selection programs for the milling industry as the groat is the fraction used for human consumption.

The hullability is the ease with which the husk is removed to get the kernel/groat. This parameter is highly important as it affects the efficiency with which the oats are milled without causing groat breakage which would result in economic losses. It is influenced by the method and conditions of dehulling used and the different size of the grain (White and Watson, 2010).

All kernel content and hullability determinations were assessed using 30 g of each sample using a *Codema impact dehuller*; Model LH5095 (set at 100 bar for 45 seconds), and then separating the output into husks, groats and whole grain. Each fraction obtained was weighed using a precision scale and the kernel content and hullability determined using the equations below.

$$KC = \left(\frac{Groat\ weight}{Initial\ weight - Whole\ grain}\right)\ x\ 100 \tag{3}$$

$$Hullability = \left(100 - \frac{Groat}{Initial\ weight}\right) x\ 100 \tag{4}$$

2.2.5 Grain and groat size and shape

Physical analysis of grain size and shape, including area, length and width of the grain and the groats once they were dehulled, were determined by a non-destructive method, using a Digital Seed Analyser, *MARVIN* (GTA Sensorik GmbH). The same 30 g sample that was used for thousand grain weight, kernel content and hullability determination was used at all times. Seeds were placed on the analysing tray and spread out so that no seeds were touching. All seeds in the sample were measured requiring several scans with MARVIN. Special software evaluated the captured image on the basis of digital image processing. The output gave the number of seeds analysed and the individual grain length, width and area. The grain sample was then dehulled and the process repeated with the groats.

Grain and groat area, length and width, were also used to determine shape descriptors as described below:

$$Ratio\ of\ the\ Grain\ or\ Groat = \left(\frac{Width\ of\ the\ grain\ or\ groat}{Length\ of\ the\ grain\ or\ the\ groat}\right) \tag{5}$$

$$Circularity = \sqrt{\left(\frac{Area\ of\ the\ grain\ or\ the\ groat}{\pi\ x \left(\frac{Length\ \text{of the grain or groat}}{2}\right)2}\right)} \tag{6}$$

$$Grain\ or\ Groat\ Density = \left(\frac{Thousand\ grain\ weight\ of\ the\ grain\ or\ the\ groat}{Area\ \times Width\ of\ the\ grain\ or\ the\ groat}\right) \tag{7}$$

Other determinations and shape descriptors will be explained in detail in the appropriated chapter where they are calculated.

2.2.6 Bimodality

In addition to the mean grain length, width and area of each sample, the individual grain and groat data were analysed to establish the frequency of the distribution of the grain population according to those dimensions. Where appropriate, this included determination of the bimodality of the population of grains analysed. Grain and groat size parameters were considered to be a mixture of two normal distributions.

$$d = v \int_{npd} (\mu_1, \sigma_1) + (1 - v) \int_{npd} (\mu_2, \sigma_2)$$

Where μ is the mean and σ the standard deviation of the normal probability density function ($\int_{npd}$) for the component distributions (subscripts 1 and 2) and *v* is the proportion in population 1 (Wychowaniec, Griffiths, Gay, and Mughal, 2013).

The bimodal distribution was fitted iteratively with initial values for μ_1 and μ_2 set to 25% (μ_1) and 75% (μ_2) quartiles of the overall distribution of grain size (x). Initial values for σ_1 and σ_2 were both set to $\surd\ (var\ (x) - 0.25(\mu_1 - \mu_2))^2$ where *var (x)* is the variance of *x* , and *v* was always set to 0.5 (Alan Gay, personal communication)

A Matlab script (MathWorks, 2013) was used to find the maximum likelihood estimation of means and variances of each distribution. Comparative graphical analysis is presented at each chapter where this analysis was performed. Violin plots were developed in R for graphical representation (courtesy Moron-Garcia, Odin). Violin plots are similar to box plots, except that they also show the probability density of the data at different values in the simplest case this could be a histogram. Overlaid on this box plot is kernel density estimation. Like box plots, violin plots are used to represent comparison of a variable distribution or sample distribution.

2.2.7 Grain composition

Approximately 20 grams of each sample of husked oats and whole groats were scanned at 2 nm intervals over the wavelength range from 400 to 2498 in reflectance mode, by a FOSS NIR (Near-Infrared Spectroscopy), Systems 6500 spectrophotometer, a non-destructive technique. NIR uses an electromagnetic spectrum that implies the vibrational response of molecular bonds O-H, C-H, C-O and N-H, and the specific vibration pattern in these bonds. Biological molecules present within these bonds, e.g. oil, protein, starch and fiber, absorb vibrational energy in a specific way generating a characteristic spectrum that behaves as a fingerprint of the sample (Bokobza, 1998). Husked and dehulled oats were scanned at 2nm intervals over the wavelength range from 400 to 2498 in reflectance mode, by a NIR (Near-Infrared Spectroscopy) (Bokobza, 1998). The general method consists in spectral data acquisitions, data pre-processing to reduce the noise and baseline shift from the instrument and the background, to build the calibration models using samples of known concentration by well referenced methods and finally validate the model. Quantification of oil, protein, β-glucan were determined using a calibration curve developed internally at IBERS and built up on the basis of the analysis of spring and winter oat samples harvested between 1998 and 2016. Wet chemistry analyses were completed on selected samples to validate the NIR screening. Samples were presented as whole oat (dried and undried) and milled (dried and undried). Calibrations were developed using modified partial least squares (MPLS) regression plus scatter corrections applied. Equations were developed using standard normal variate and detrend (Dhanoa, Barnes & Lister, 1989) and second derivative transformations using modified partial least squares (mPLS) regression. The methods used to select samples for equation update are described in Shenk and Westerhaus (1991). It included total N analysis on ground groat samples which was performed using the Kieldahl method (AOAC method 945.18) (199) using a LCEO FL-48 analyser (LECO Corp, ST. Joseph, MI). Oil calibration data was obtained by extraction using petroleum ether and the Soxtec system (FOSS UK, Warrington, UK). The β-glucan content was determined in parallel using the Megazyme ™ kit (McCleary method AOAC method 995.16) on all samples (Megazyme and Ireland, 1991). NIR scans from the whole oats were used to develop a calibration for kernel content.

2.2.8 Statistical analysis

In each chapter, to check and summarize dataset characteristics the mean, standard deviation and standard error of the mean of each trait, were calculated according to the factors, i.e. variety, site, fertilizer level, or harvest season, involving the experimental design using Genstat 2013 and Excel 2013. Correlations were calculated using the means in every field season and site. Genstat 2013 was used to calculate the correlations. Graphs were drawn using Excel 2013 and R studio.

All the specific statistical methods were chosen according to the statistical requirements and distribution characteristics. These included: two-way ANOVA with variety and site as factors, to determine the significance of both; Pearson´s correlations between all traits under study, by each of the factors implied; Joint regression analysis (Finlay & Wilkinson, 1963), superiority performance and the stability coefficient, i.e. genotypes´ consistency in responding to changes in the environment (Lin & Binns, 1991a). Specific statistical analysis developed further is explained in detail in each chapter.

Chapter three. Genotype by Environment study

3.1 Introduction and analysis of historical data.

The actual challenge for the cereal market, including the oat market, is the necessity to increase grower returns whilst minimising environmental impact. Grain yield and quality determine the value of an oat crop to the producer. The most common quality measurement used is test or specific weight (see Introduction chapter for a definition). However, it is not a measurement related with any processing trait, and it is not good predicting milling yield (White et al., 2003). Other grain quality traits, i.e. kernel content, thousand grain weight, hullability and grain composition (β-glucan and protein and oil content), are highly desirable for the milling industry, human consumption and for animal feed but these traits are more laborious to measure. Whilst specific weight can be measured easily and quickly in the field, kernel content, hullability and grain chemical composition requires technology, e.g. MARVIN, NIR, technical skills and time (see Material and Methods).

Compared with other cereals e.g. wheat and barley (Clarke, Gooding & Jones, 2004; Hundal, Kang & Singh, 2017; Lehmensiek, Sutherland & McNamara, 2008; Ma, Biswas, Zhou, & Ren, 2012; Paroda & Hayes, 1971; Pushman & Bingham, 2017), knowledge of genotype by environment effects on grain quality parameters are more limited, partly because of less research and funding, leading to a poorer understanding of the mechanisms underlying grain quality traits (Cooper, 1937). Previous studies have shown conflicting results, in terms of the effect that both, the genotype and the environment and their interaction have on grain quality traits (Doehlert, McMullen & Hammond, 2001; Peterson et al., 2005). Therefore, while some results suggest that major variation in specific weight can be explained by variety choice, other researchers have found equal effects from both environment and genotype. These confounding results make more difficult selection and development of new varieties.

To identify the variability that exists for grain quality parameters and yield across environments and years, historical data was obtained from the AHDB recommended list trials from 2008 till 2013. This allowed the evaluation of the varieties' performances from standardised field trials performed in a range of locations across the U.K. ("AHDB Cereals &

Oilseeds: Current trials and harvest results," 2008/13). These trials are conducted independently each year at a range of sites that represent oat growing areas and are used to both identify superior new varieties and to provide data for end-users such as farmers to select suitable varieties for their purposes. Successful new varieties must not only have high grain yield and quality but also perform well over a wide range of environments.

Recommended list oat varieties usually reflect average values for specific quality parameters from the latest harvest season. These average values are obtained when possible, from each site where the AHDB is conducting trials, i.e. some trials and years are lacking some quality parameters measurements.

Table 3.1. *Mean yield (t/ha), Grain quality and agronomic values of four winter oat varieties used in this research, from 2008 to 2013. Data extracted from Recommended List* (AHDB Cereals & Oilseed, 2008-13).

** = variety no longer in trial from 2012. C = yield control, Gerald from 2008 to 2013 and Mascani for 2012/2013 harvest season. All relative yields from 2008 to 2013 on this table are taken from treated trials receiving a full fungicide programme. On the 1-9 scales high figures indicate that a variety shows the character to a high degree (e.g. disease resistance). # The winter hardiness is measured on a scale where scores above 5 indicate only leaf damage and no plant death.*

Quality Traits	Tardis *	Gerald C	Mascani C	Balado
Scope of Recommendation	**UK**	**UK**	**UK**	**UK**
UK yield as % treated control (8.3 t/ha)				
Fungicide treated	**103**	**99**	**99**	**105**
Untreated with fungicide	**96**	**89**	**95**	**101**
Grain quality				
Kernel content (%)	**73**	**73**	**78**	**73**
Specific weight (kg/hl)	**50**	**53**	**54**	**50**
Screenings % through 2.0mm	**0**	**1**	**0**	**1**
Agronomic features				
Resistance to lodging	**7**	**6**	**6**	**7**
Straw length (cm)	**111**	**119**	**117**	**100**
Ripening (days +/- Gerald, -ve = earlier)	**-2**	**-1**	**-1**	**-1**
Winter hardiness #	**8**	**8**	**7**	**8**
Disease resistance				
Mildew	**8**	**5**	**6**	**6**
Crown rust	**7**	**4**		**5**
Year first listed	**2007**	**1993**	**2004**	**2010**
Treated yields with and without PGR as % treated control				
With PGR (8.2 t/ha)	**101**	**98**	**98**	**106**
Without PGR (8.1 t/ha)	***106***	***101***	***101***	***112***

The varieties listed in table 3.1 and 3.2 include the four winter oat genotypes from the Aberystwyth University winter oat breeding programme that will be under study in this chapter, i.e. Balado, Gerald, Tardis and Mascani. The data available included among others, grain yield, specific weight and kernel content. Due to the progression of old and new varieties onto and off the recommended list (table 3.2), not all varieties were tested in all years and some missing data was present in the data supplied by AHDB. Therefore, a complete statistical analysis comparing both as factors was not conducted and a graphical analysis was applied to the dataset.

Table 3.2. *Average values of lodging (%), height (cm) and ripening days, for the four winter oat varieties, Balado, Gerald, Mascani and Tardis from 2007 to 20013. Data extracted from Recommended List* (AHDB Cereals & Oilseed, n.d.)*. N/a data not available as variety not on recommended list.*

Years	Total Sites	Lodging %				Height cm				Ripening Days			
		Balado	Gerald	Mascani	Tardis	Balado	Gerald	Mascani	Tardis	Balado	Gerald	Mascani	Tardis
2007	39	N/A	21.3	19.2	20.4	N/A	121.8	117.1	114.1	N/A	310.3	310.5	310.2
2008	28	3.3	33.3	33.3	33.9	87.4	110.9	99.7	98.7	292.0	290.7	286.0	288.0
2009	55	0.0	15.0	0.0	0.0	88.3	106.3	102.3	96.9	293.1	292.9	291.8	289.2
2010	68	0.0	6.7	3.7	3.0	97.9	120.5	115.5	109.5	312.4	312.4	312.0	311.8
2011	52	0.0	1.3	1.3	1.7	74.8	93.7	91.9	88.9	286.8	281.7	281.5	282.0
2012	30	0.6	2.8	0.0	N/A	102.1	119.3	116.9	N/A	317.5	317.2	315.8	N/A
2013	21	0.0	0.0	0.0	N/A	79.1	96.2	95.0	N/A	291.0	291.0	291.0	N/A

Although the overall performances of each of the varieties provides a guide to their quality, deconstructing the mean by year and variety, allows the variability between years and within years between sites (table 3.2) to be investigated. The number of sites tested each year, and the mean for each variety on a yearly basis, regarding lodging (%), height (cm), are indicated in table 3.2, whilst yield (t/ha), kernel content (%) and specific weight (kg/hl) average by year and variety, are represented in the figures 3.1 to 3.6.

The average yield by year, from 2008 to 2013, for four winter oat varieties, Balado, Gerald, Mascani and Tardis is shown in figure 3.1. Although 2009 was the highest in terms of yield, both, specific weight (figure 3.2) and kernel content (figure 3.3), were not as high as in

2008. Mean yields and specific weights were lowest in 2012 whereas mean kernel content was highest in 2011 and lowest on 2010. This variability was found for all spring and winter oat variety results from recommended list trials (data not shown). Considerable variation between years was found for all traits reported.

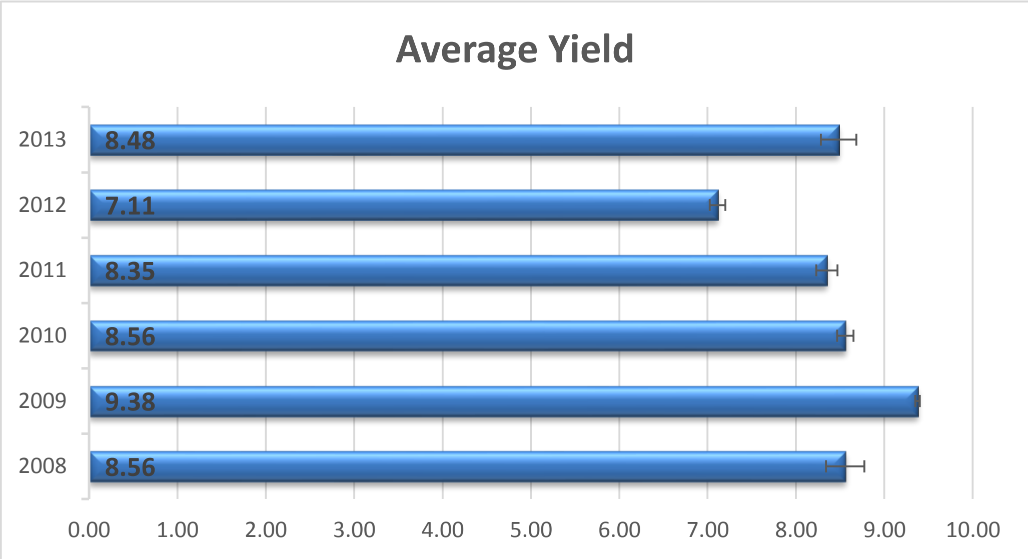


Figure 3.1. *Average yield (t/ha) ± s.e.m. value by year for the four winter oat varieties shown in table 1. Data from historical reports of recommended list trials. AHDB personal communication.*

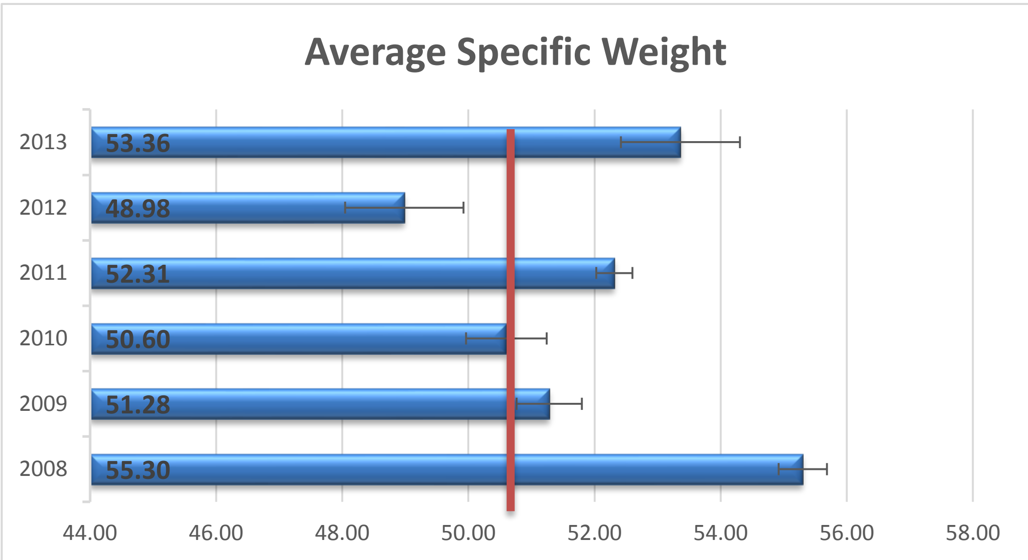


Figure 3.2. *Average Specific Weight (kg/hl) ± s.e.m., for four winter oat varieties, Balado, Gerald, Mascani and Tardis, from 2008 to 2013. The red line represents the minimum value for a variety to be included on the recommended list at the time of testing (50 kg/hl). Data from historical reports of recommended list trials. AHDB personal communication.*

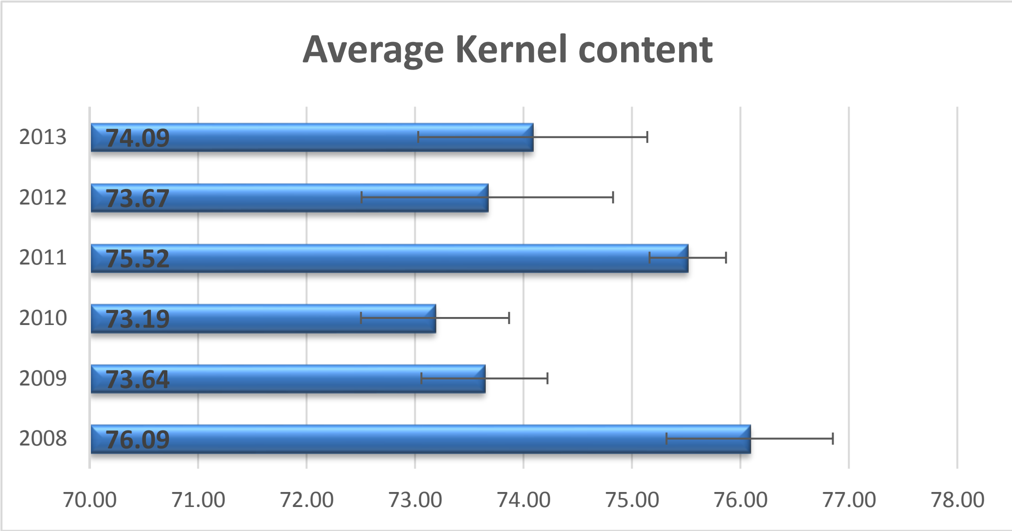


Figure 3.3. *Average kernel content (%) ± s.e.m., for four winter oat varieties, Balado, Gerald, Mascani and Tardis, from 2008 to 2013. Data from historical reports of recommended list trials. AHDB personal communication.*

If the mean grain yield of specific varieties from 2008 to 2013 is examined (figure 3. 4), they are, graphically speaking, quite similar with 75% of the results between 8 and 10 t/ha for all varieties. This stability might be explained given the complexity of this trait, with not only one model explaining its components (Adams & Grafius, 1971). Both, specific weight, (figure 3.5) and kernel content (figure 3.6) were, graphically speaking, different between the four varieties. For a variety to be added to the recommended list it must meet certain criteria including a minimum specific weight of 50 kg/hl. Balado presented the highest levels of variability in terms of specific weight, with values under market requirements in 2010 and 2012, despite having a good yield in almost all years. Tardis showed a similar performance with the specific weight average values falling below 50 kg/hl in 2009 and 2010, but a more consistent outcome in terms of kernel content and yield was found. Mascani and Gerald were more consistent between years and were above the minimum required for all traits under study.

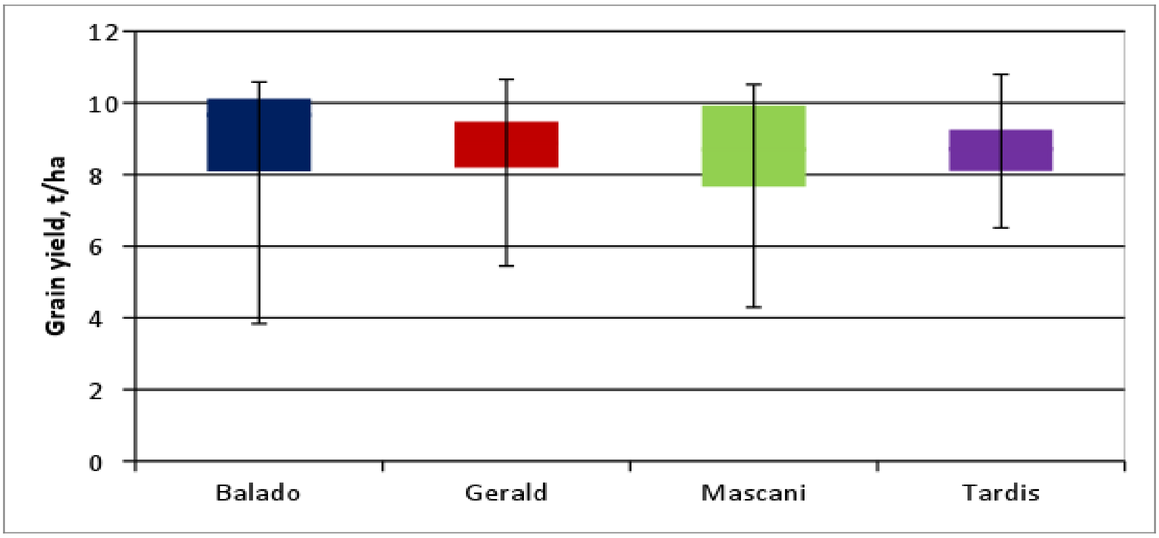


Figure 3.4. *Box plots indicating the average yield values (t/ha) ± s.e.m. for four winter oat varieties, i.e. Balado, Gerald, Mascani and Tardis, from 2008 to 2013. AHDB historical reports (personal communication).*

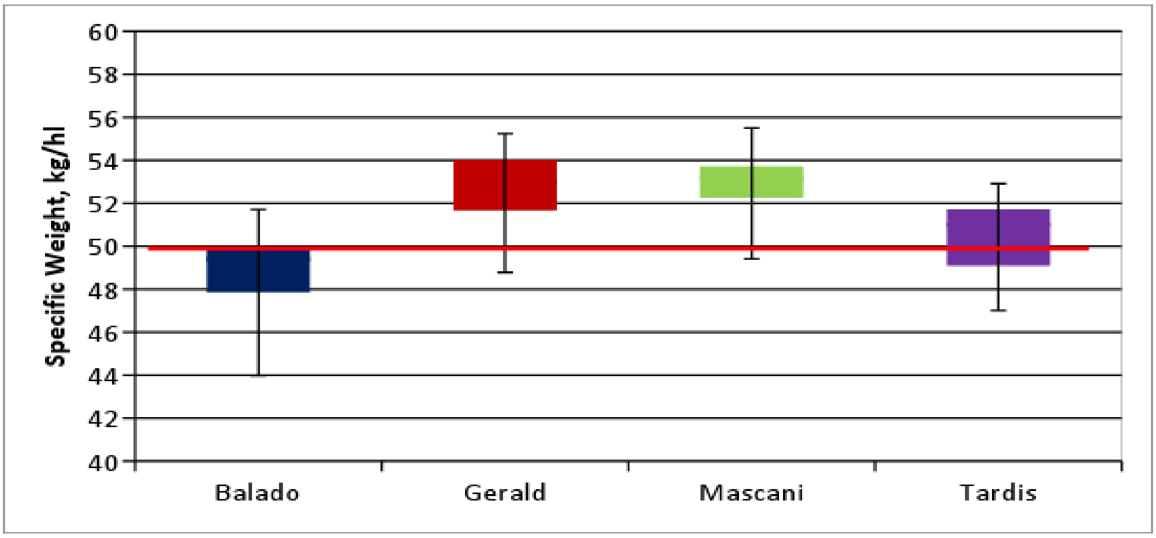


Figure 3.5. *Box plots indicating average specific weight values (kg/hl) ± s.e.m., for four winter oat varieties, i.e. Balado, Gerald, Mascani and Tardis, from 2008 to 2013. The red line represents the minimum value accepted in the milling industry (50 kg/hl).*

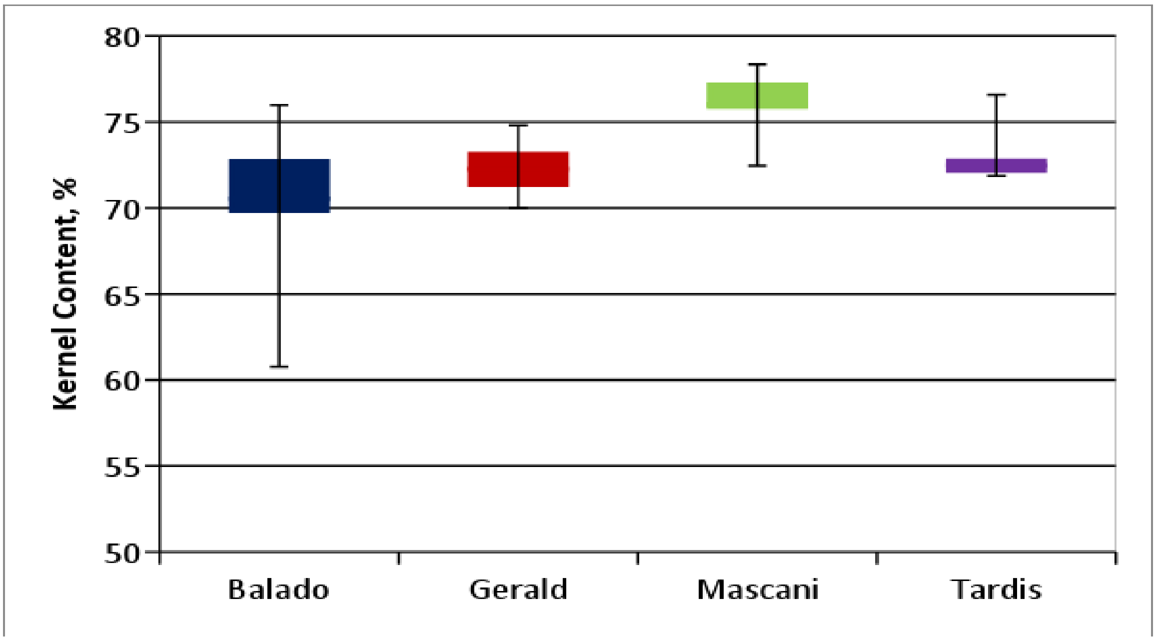


Figure 3.6. *Box plots showing average kernel content values (%) ± s.e.m. from 2008 to 2013, of four winter oat varieties, Balado, Gerald, Mascani and Tardis. Data from AHDB historical reports (personal communication).*

The variability in grain quality that is evident in figures 3.4 and 3.5 might be explained by both genetic differences between varieties and their interactions with the environment. Having established that considerable variation for grain quality traits is present not only between varieties but also across years, this chapter describes the results from multi-site replicated field trials across the major areas of oat production in the United Kingdom (figure 3.7) using the four varieties indicated in table 3.1 and 3.2.

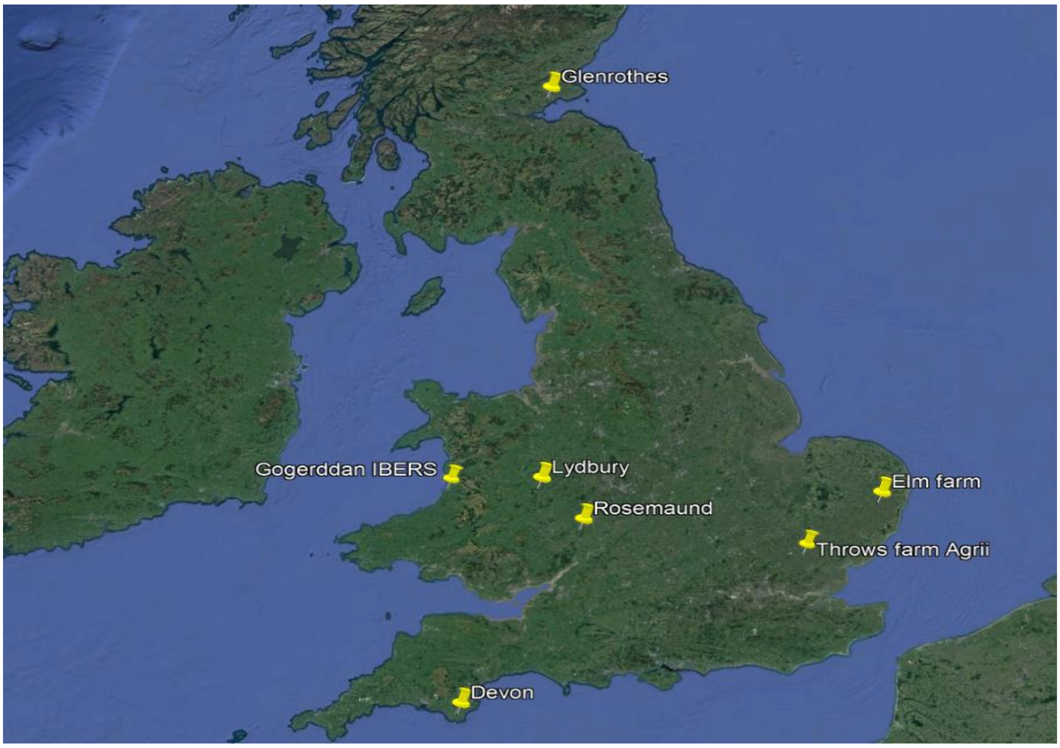


Figure 3.7. *Field trials sites across the country in 2012-2013 and 2013-2014 harvest seasons.*

The objective of this study was first, to establish the genetic differences between varieties and the effect of different environmental growing sites on grain quality parameters and yields. Secondly, to determine whether there are genetic and environmental interactions for grain quality traits under study. Thirdly, whether there is any kind of relationship between grain quality parameters. By the non-destructive analysis of grain, the physical basis that determines grain quality parameters in oats was dissected.

A clear knowledge and understanding of the relationship between the genetic factors and the environment will benefit variety selection methods in breeding programs. This knowledge will have an important economic impact for the milling industry, accelerating selection methods of variety breeding, and focusing grain quality traits through the development of new and more suitable varieties of oats. For arable producers, it will help to develop agronomic practises that maximise the use of land and diminishing environmental impact due to crop production and fertilization methods, balancing high yield and milling grain quality.

3.2 Plant material. Experimental design and methods.

3.2.a. Details of field trials

The four winter oat varieties were grown in replicated field trials at 11 sites across the United Kingdom (figure 3.7, table 3.3) over two harvest years (2012/2013 and 2013/2014). Sites were chosen to represent contrasting environmental conditions within the UK and included the geographical areas where oats are grown in arable rotations

Table 3.3. *Site codes, longitude and latitude, site codes, sowing dates and harvest dates at each site. The site codes were assigned to identify, graphically, the site within each year where samples were taken to analyse for the present research.*

Site	*Site code*	*Longitude/Latitude*	*Year*	*Sowing date*	*Harvest date*
Gogerddan	1	-4.02/52.43	2013	23/10/12	18/8/2013
Glenrothes	2	-3.11/56.19	2013	2/10/2012	14/8/2013
Devon	3	-3.76/50.27	2013	20/10/2012	13/8/2013
Rosemaund	4	-2.39/52.09	2013	6/2/2013	3/9/2013
Elm farm	5	1.35/52.36	2013	16/10/2012	24/8/2013
Gogerddan	6	-4.02/52.43	2014	25/9/2013	24/7/2014
Lydbury	7	-2.94/52.45	2014	8/10/2013	20/8/2014
Glenrothes	8	-3.11/56.19	2014	26/9/2013	4/8/2014
Devon	9	-3.76/50.27	2014	7/10/2013	31/7/2014
Rosemaund	10	-2.39/52.09	2014	30/9/2013	31/7/2014
Throws farm	11	0.41/51.58	2014	5/10/2013	22/7/2014

Each trial included at least three replicate plots (1.8 x 6 m) of each variety, sown in a randomised block design, planted at a sowing rate of 300 seeds m^2. Fertiliser application to the seedbed and top dressing applied were according to the established protocols used for Recommended List testing of varieties in the UK considering previous crop, type of soil and levels of nitrogen present in the soil ("Section 4 Arable crops Nutrient Management Guide (RB209)," 2016) except for site 5. Site 5 was grown at an organically managed site as described in Fradgley *et al.*, (2017). Grain from each replicate at each site was harvested using a combine and subsampled for analysis in this study. Traits measured included specific weight (t/hl), kernel content (%), hullability (%), thousand grain weight (g), yield (t/ha), grain number, oil, protein and b-glucan content (%), and grain and groat size and shape using methods described in chapter 2.

3.2.b. Statistical analysis

The mean and the standard error of the mean of each trait were calculated for each variety at each site along with the overall mean for each site, by harvest season. The statistical methods were chosen to be suitable to study an unbalanced experimental design where the number and location of sites used for field trials may differ between seasons. These included: two-way ANOVA with variety and site as factors, to determine the significance of both and Pearson´s correlations between all traits under study, by both site and variety.

To evaluate the stability of a genotype across environments, a number of different indices were compared, including joint regression analysis (Finlay & Wilkinson, 1963). In this analysis a modified joint regression was performed on data classified by two factors, i.e. variety and environments, at which experiments were grown. The regression, following therefore, a non-linear model (equation 1), characterizes the sensitivity or inversely, the stability, of each variety to environmental effects.

$$y_{ij} = v_i + b_i \, x \, e_j + error \quad (1)$$

where v_i are variety means, e_j are environment effects and b_i are the sensitivity parameters or the slope of the regression.

The analysis fits a regression of the environment means for a variety on the average environment means. The regression slope (b_i) describes the general response pattern among all cultivars. $b_{i,}$ less than 0.7 means that the cultivar is better adapted to low-yielding locations, whilst b_i above 1.3 means that the cultivar is better adapted to high yield locations. Therefore, high values of b_i reflect high sensitivity to the environment whereas low values of b_i indicate that a variety is less affected by the environment.

In addition, three non-parametric measures were calculated to determine the effect of genotype, environment and their interaction. These were cultivar superiority, static stability and sensitivity (Huehn, 1990). This enabled the assessment of the stability of each variety for all the traits under study and to determine the existence of local adaptation.

Cultivar superiority (P) (equation 2) (Lin & Binns, 1991a, 1991b) measures the mathematical distance, i.e. difference, between the cultivars response and the maximum response averaged over all locations. The maximum response is the upper boundary in each location and therefore small values imply the closeness of the trait for the corresponding genotype to the maximum and therefore, a superior overall response.

$$P_i = \sum (X_{ij} - M_j)^2 / (2n) \qquad (2)$$

Where P_i represents the superiority measure of the i_{th} test cultivar, X_{ij} represents the yield of the i_{th} cultivar grown at the j_{th} location and M_j is the maximum response among all cultivars in the j_{th} location. It can be defined as the mean square of the difference between the i_{th} cultivar and the maximum responses. Since P_i is measured over all locations, it represents superiority in the sense of general adaptability.

Static stability (Lin & Binns, 1991a, 1991b) defines a stable genotype as one that possesses an unchanged performance regardless of any variation of the environmental conditions, i.e. its variance between its means in the various environments is zero. It provides a measure of the consistency of the genotype, but without taking account of how good it is.

When looking at the non-parametric stability parameters mentioned above, and joint regression sensitivity values, the mean deviations for the observations about the line fitted for each genotype were also considered. A genotype with smaller mean square deviations gives the more predictable responses (Finlay & Wilkinson, 1963).

The relative performances of each cultivar at each site were also determined by removing the effect of the environment. This was done by subtracting the mean over all genotypes at each site from the mean of each genotype at that site (Mcdermott & Coe, 2012). This allows a graphical representation of the relative performance of the genotypes at each site, removing environment variation, and therefore, enables to see which environments really discriminate between genotype performances.

To complete the analysis, the bimodality of the individual grain size traits was determined following frequency distribution analysis. Grain size parameters were considered mixture of two normal distributions (Symons & Fulcher 1988). A MATLAB script (MathWorks, 2013) was used to find the maximum likelihood estimation of means and variances of each distribution. In addition to the mean grain length, width and area of each sample, the individual grain and groat data were analysed to establish the frequency of the distribution of the grain population according to those dimensions. Where appropriate, this included determination of the bimodality of the population of grains analysed. Grain and groat size parameters were considered mixture of two normal distributions.

$$d = v \int_{npd} (\mu_1, \sigma_1) + (1 - v) \int_{npd} (\mu_2, \sigma_2) \quad (3)$$

Where μ is the mean and σ the standard deviation of the normal probability density function ($\int_{npd}$) for the component distributions (subscripts 1 and 2) and v is the proportion in population 1 (Wychowaniec *et al.*, 2013).

The bimodal distribution was fitted iteratively with initial values for μ_1 and μ_2 set to 25% (μ_1) and 75% (μ_2) quartiles of the overall distribution of grain size (x). Initial values for σ_1 and σ_2 were both set to $\surd\ (var\ (x) - 0.25(\mu_1 - \mu_2))^2$ where *var (x)* is the variance of *x* , and *v* was always set to 0.5 (Alan Gay, personal communication)

A MATLAB script (MathWorks, 2013) was used to find the maximum likelihood estimation of means and variances of each distribution. Comparative graphical analysis is presented at each chapter where this analysis was performed.

3.3 Results

3.3.1 Weather conditions.

Autumn 2012 was wet leading to difficult planting conditions (figure 3.8). Overall, 2013 was characterized by exceptionally cold spring, leading into a warm and sunny summer ('Met Office', 2015). The mean summer temperature was 0.8 C above the 1981-2010 average, the summer rainfall total was 187 mm (78% of average), and the summer sunshine total was 578 hours. In 2014, the winter was warm and wet (rainfall 165% of average) leading into a warm but wet spring and a sunny summer (113% of average).

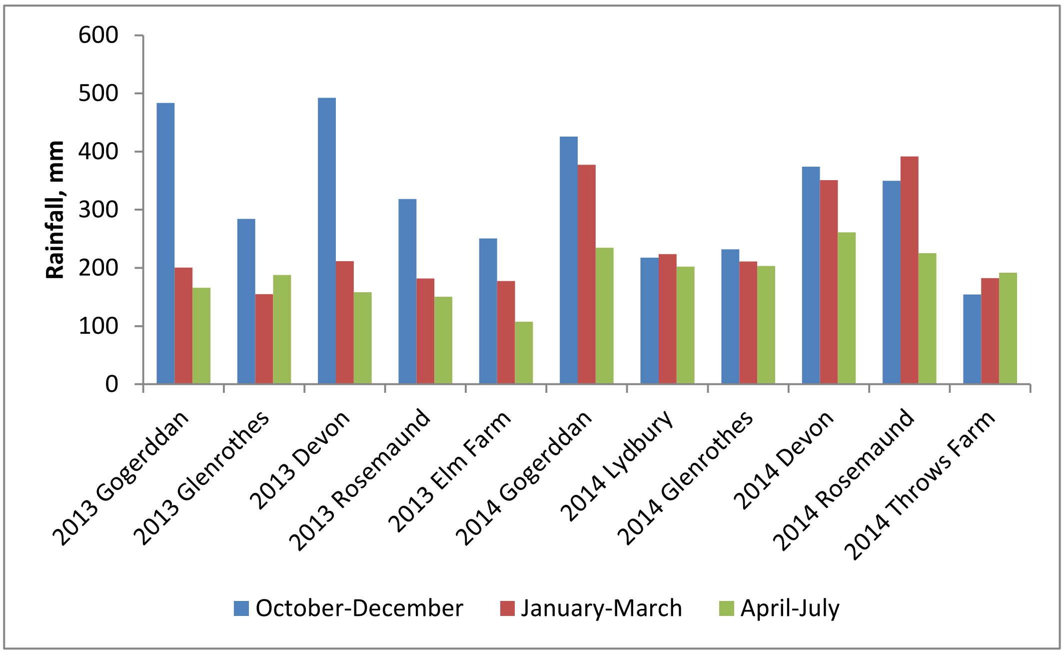


Figure 3.8. *Average rainfall (mm) values at each of the eleven sites across UK during both harvest seasons, 2012/2013 and 2013/2014, used in this research.*

Detailed weather data (daily maximum and minimum temperature (^{o}C), rainfall (mm) and relative humidity (%)) were only available from Gogerddan site for both the 2012/2013 and 2013/2014 harvest seasons. From the temperature data, Growing degree days (GDD) were calculated using as 0 ^{o}C as the base temperature (figure 3.9). The data from both harvest seasons coincide in the amount of days between sowing and harvesting dates, giving a total of 302 days for both seasons. However, the curve of GDD (figure 3.9) shows the difference in the amount of thermal time accumulated in the two seasons. In 2013/2014, daily mean temperatures were higher in the autumn and summer than in 2012/2013. At the same time, cumulative rainfall during the season was similar for the first 120 days in 2012/2013 and 2013/2014 at Gogerddan but thereafter it was much drier in 2012/2013 (figure 3.10). Although other weather parameters need to be considered, such as humidity, wind as well as previous soil conditions and crop, these differences might explain some results obtained in terms of grain and groat quality parameters.

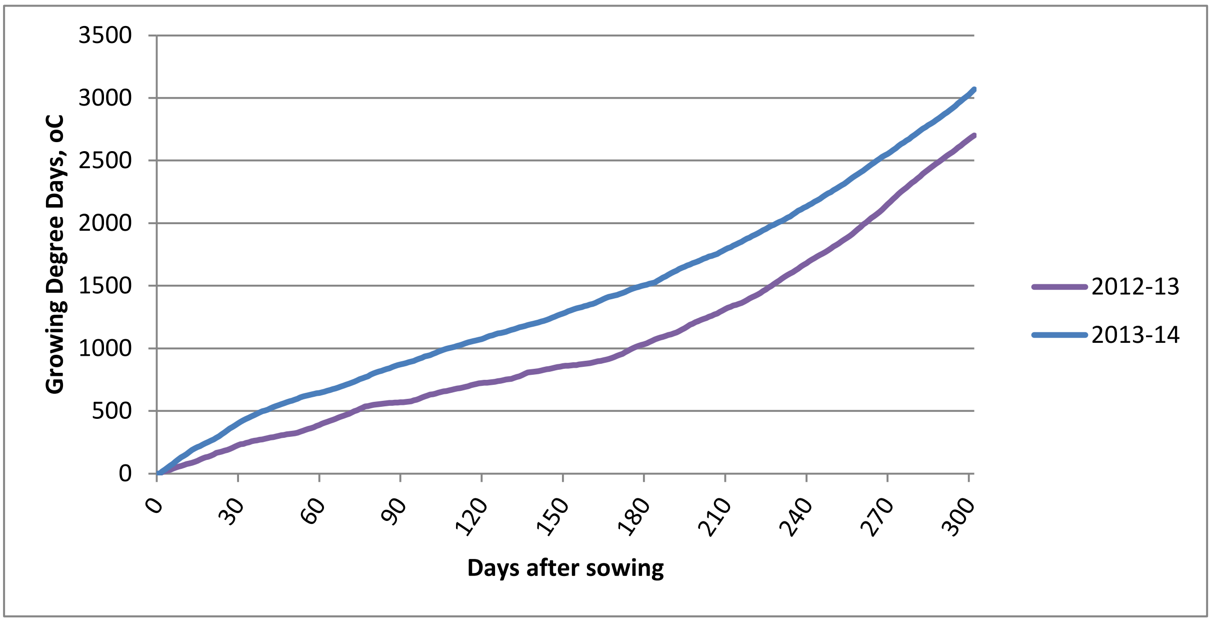


Figure 3.9. *Growing Degree Days (GDD) ºC, at both 2012/2013 and 2013/2014 harvests seasons, for Gogerddan (Catherine Howarth, personal communication).*

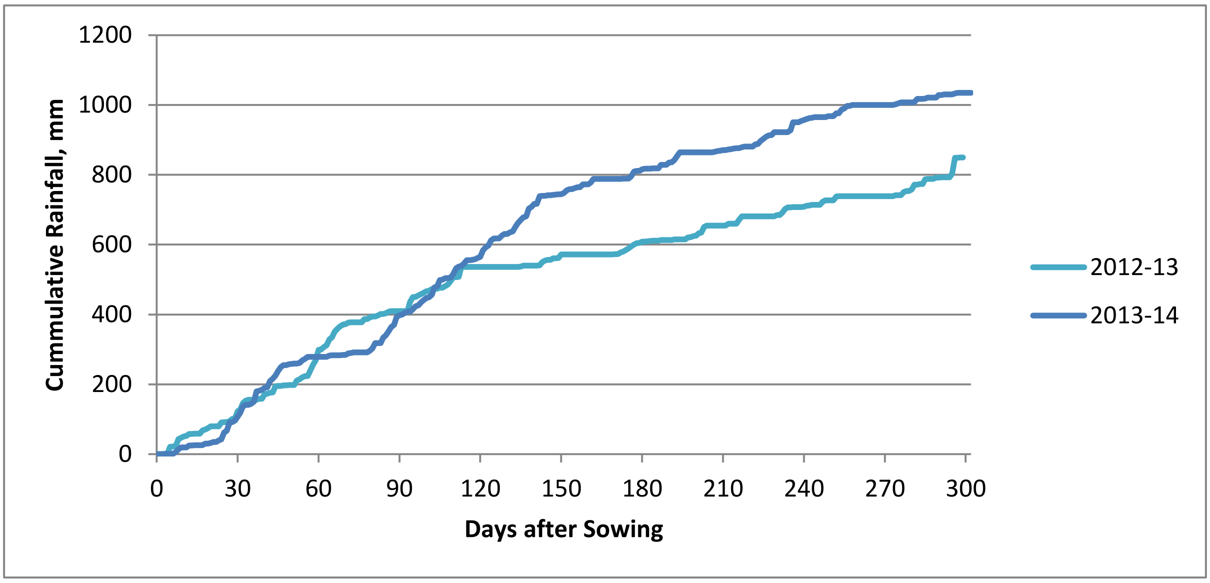


Figure 3.10. *Cumulative rainfall (mm) at both 2012/2013 and 2013/2014 harvests seasons, for Gogerddan (Data from Gogerddan met station).*

Table 3.4. Effect of the environment on the mean ± s.e.m. values for yield (t/ha) kernel content (%), specific weight (kg/hl), yield (t/ha), thousand grain weight and grain number per meter square. of four winter oat varieties at 11 all sites and both harvest seasons.

Site	*Site code*	*Year*	*Yield (t/ha)*	*s.e.m.*	*Grain nº/m²*	*s.e.m.*	*Kernel content (%)*	*s.e.m.*	*Specific weight (kg/hl)*	*s.e.m.*	*Hullability (%)*	*s.e.m.*	*Thousand Grain Weight (g)*	*s.e.m.*
Gogerddan	1	2013	8.3	0.1	19846.0	237.6	73.1	0.2	50.2	0.3	77.5	1.6	42.0	0.3
Glenrothes	2	2013	8.8	0.1	21900.7	276.7	73.1	0.2	52.1	0.2	84.9	1.1	40.2	0.3
Devon	3	2013	10.5	0.1	26824.1	309.7	72.2	0.3	51.8	0.2	75.9	1.6	39.4	0.3
Rosemaund	4	2013	5.0	0.1	12622.1	422.8	75.7	0.2	50.8	0.1	92.4	0.7	40.6	0.4
Elm farm	5	2013	9.7	0.1	23142.1	279.6	72.7	0.2	51.9	0.3	76.3	1.6	42.1	0.4
Gogerddan	6	2014	9.3	0.1	24630.7	387.8	69.7	0.7	50.3	0.3	84.1	1.3	38.5	0.7
Lydbury	7	2014	7.9	0.1	16514.3	213.3	75.2	0.2	53.7	0.2	91.4	0.7	48.2	0.4
Glenrothes	8	2014	9.7	0.1	22457.2	178.3	72.9	0.3	53.2	0.2	80.6	1.3	43.2	0.3
Devon	9	2014	9.9	0.1	22789.2	312.5	72.8	0.3	50.9	0.3	83.7	1.2	43.7	0.3
Rosemaund	10	2014	7.1	0.1	17227.6	227.5	73.9	0.3	49.2	0.2	86.9	0.9	41.5	0.4
Throws farm	11	2014	9.4	0.0	26769.6	318.0	70.5	0.5	49.2	0.4	83.1	1.1	35.4	0.4
Overall mean			8.7	0.9	21338.5	590.3	72.9	1.6	51.2	1.1	83.3	2.0	41.3	0.9
Significance Site			<0.05		<0.05		<0.001		<0.001		<0.001		<0.001	
Significance Genotype			Non significant		<0.05		<0.001		<0.001		<0.001		<0.001	
Significance Interaction			<0.05		Non significant		<0.001		Non significant		<0.001		<0.001	

3.3.2 Yield

Analysis of variance (two way ANOVA) showed significant differences (p-value<0.05) for grain yield between sites. The lowest average value 5.0 t/ha (table 3.4) was obtained at Rosemaund 2013 (site code 4), whilst the Devon 2013 (site 3) yielded the highest value of 10.5 t/ha (table 3.4, figure 3.11). The overall average value was 8.7 t/ha. At the same time, there were significant interactions between environment and variety (p-value<0.05, two-way ANOVA). However, there were no significant differences between varieties (table 3.5). Grain number per m^2 also was significantly different (p-value<0.05) between sites and between varieties but there were no significant interactions with the environment (table 3.5).

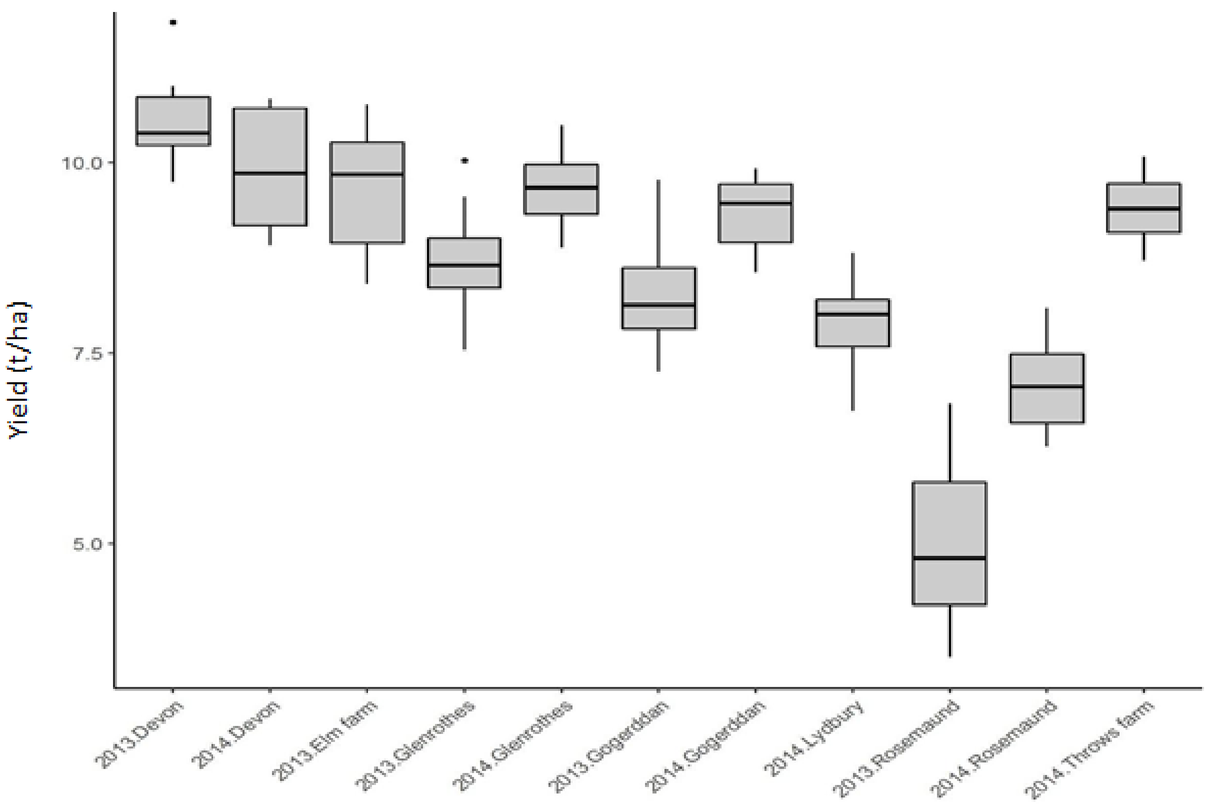


Figure 3.11. *Box plot of yield (t/ha) values of four winter oat varieties, Balado, Gerald, Mascani and Tardis, from each site and harvest season (2012/2013 and 2013/2014). The box plot* (Weisstein, 2018) *represents between first quartile (25 %) and the third quartile of the data 75 %, with the horizontal line inside the box indicating the median. The whiskers represent the data within 1.5 times the interquartile range of the first quartile and the third quartile. Data points represented by stars are outliers, i.e. they are more than farthest from 1.5 times the interquartile range of the first quartile and the third quartile.*

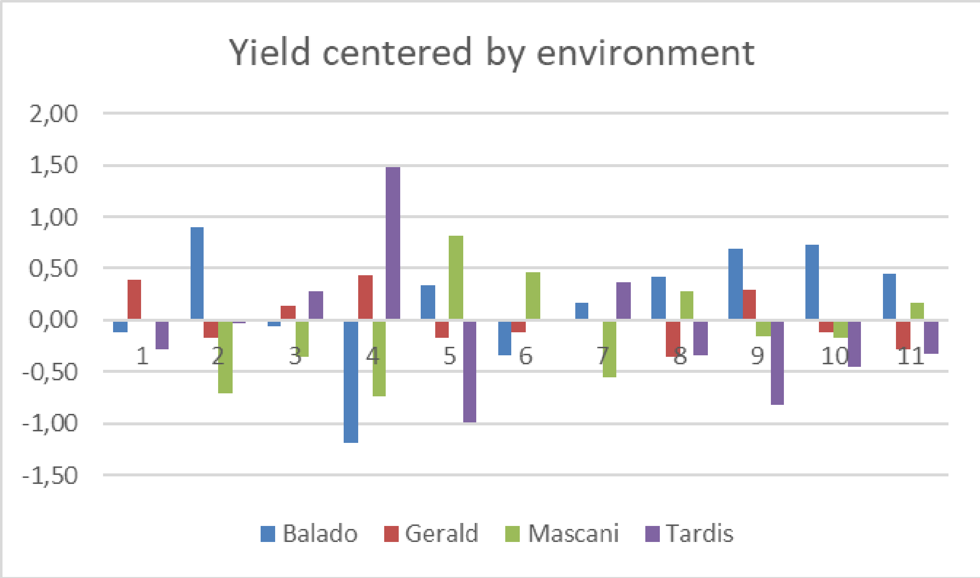


Figure 3.12. *Grain yield (t/ha) centred by environment of the four varieties, i.e. Balado, Gerald, Mascani and Tardis, at each site (see Table 3.3), for both 2013 and 2014 harvest seasons. This graph was developed by subtracting the mean yield over all genotypes at each location from the yield of each genotype at that location. This gives a mean yield at all environments of zero allowing removing the effect of the environments and comparing genotypes performances.*

The effect of the environment on each variety can be determined in several ways Figure 3.12, shows the relative performance of the genotypes at each location and removes the environment to environment variation. This visualises which genotypes are yielding above and below average at a given environment, as well as the ranking of genotypes by yield at each environment. The range of yield values found graphically, by variety, reflects the sites that would be more interesting to discriminate between varieties´ performances, i.e. to investigate further in which sites a genotype yields well, and in which it performs poorly. Therefore, site 2, Glenrothes 2013, site 4, Rosemaund 2012/2013, site 5, Elm farm 2013, site 9, Devon 2014, and site 10, Rosemaund 2014, showing a wider range of values are the best environment to discriminate between the four varieties. However, the rest of the sites did not have visible differences in the performance of the different genotypes and therefore are less useful to discriminate between genotypes. There was not a consistent effect of genotype apparent across environments and no genotype performed consistently better at all sites.

Joint regression analysis (Finlay & Wilkinson, 1963), was also used to determine phenotypic stability and the sensitivity of trait performance to the environment (figure 3.13, table 3.5). In this analysis, the variety performance is plotted against the environment mean at each site and a linear regression is performed. This regression of the genotypic response on an environmental index, such as the average of all phenotypes in an environment, is defined as the difference between the marginal mean of the environment and the overall mean. The slope of the regression line represents the sensitivity of a variety to the environment. A phenotype with a regression coefficient of 1 and minimum deviations from the regression will be considered as most stable. The general stability (Lin & Binns, 1991b), is a cultivar homeostatic ability to withstand unpredictable environmental variation.

The sensitivity and static stability values obtained (table 3.5), indicated that across environments Tardis was the more stable variety, i.e. an unchanged performance regardless of any changes on the environmental conditions, meaning its variance between environments is the closest to zero (Lin & Binns, 1991b), whereas Balado had the highest sensitivity to the environment. This shows that Gerald however was the highest in cultivar superiority ranking (table 3.5).

It is also interesting to consider the mean of the square deviations of the observations about the line fitted for each genotype. Gerald with a value of 0.219 mean square deviation (figure 3.13), is giving the most predictable responses. However, static stability values show Tardis as the genotype with an unchanged performance regardless of any changes on the environmental conditions, in other words, its variance between environments is zero.

Table 3.5. *Average yield (t/ha) over all sites, cultivar superiority, static stability and sensitivity of the four winter oat varieties. *Numbers in brackets indicated the ranking positions of each variety, as best cultivar.*

Yield t/ha	Mean	Cultivar Superiority	Static Stability	Sensitivity	Mean Square Deviation
Varieties					
Balado	8.87	0.38 (2)	3.74 (4)	1.20(4)	0.35 (4)
Gerald	8.70	0.26 (1)	2.16 (2)	0.92 (2)	0.22(2)
Mascani	8.60	0.48 (4)	3.48 (3)	1.17 (3)	0.42(3)
Tardis	8.59	0.44 (3)	1.49 (1)	0.70 (1)	0.41(1)
Significance	n.s.	*p-value<0.05*	*p-value<0.05*	*p-value<0.001*	*p-value<0.001*

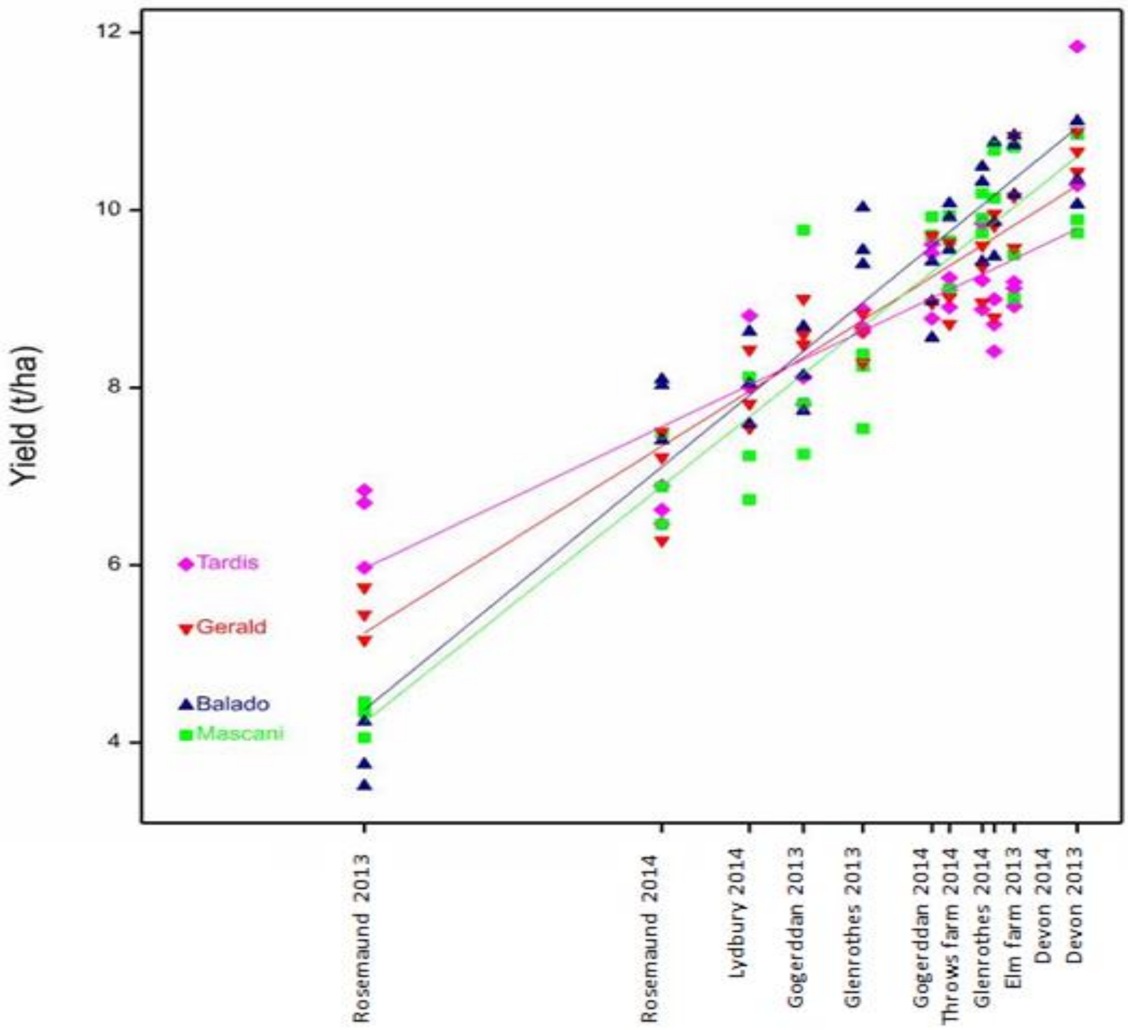


Figure 3.13. Joint regression plot (Finlay & Wilkinson, 1963), of four winter oat varieties yield performance against sites for both harvest seasons 2013 and 2014

3.3.3 Kernel content

Mean kernel content (table 4.4) was statistically significantly different (p-value < 0.001, two-way ANOVA) for varieties and sites, as well as showing a significant genetic by environment interaction (p-value <0.001).

Between varieties, Balado had the lowest mean kernel content with a value of 70.4% whilst Mascani showed the highest with 76.6% (table 4.6, figure 4.14). Interestingly, a wider range of values was found for Balado in 2014 in comparison with the 2013 harvest season (figure 4.14), while the rest of the varieties did not show differences between years. Mean kernel content (table 3.4) was statistically significantly different (p-value < 0.001, two-ways ANOVA) for both varieties and sites, showing as well as showing a significant genetic by environment interaction (p-value <0.001).

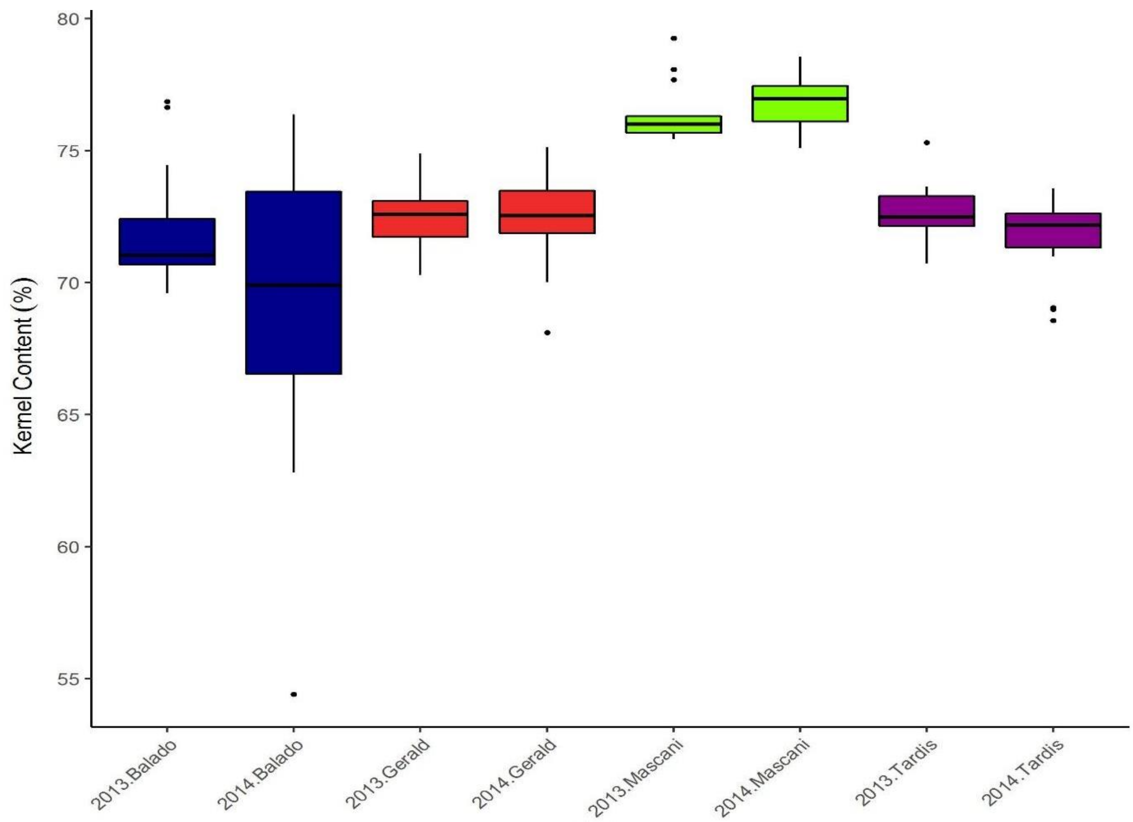


Figure 3.14. Box plot of k*ernel content (%) by variety and year of the four winter oat varieties, Balado (blue), Gerald (red), Mascani (green) and Tardis (purple), for both harvest seasons, 2013 and 2014. The box plot* (Weisstein, 2018) *represents between first quartile (25 %) and the third quartile of the data (75 %), with the horizontal line inside the box indicating the median. The whiskers represent the data within 1.5 times the interquartile range of the first quartile and the third*

quartile. Data points represented by stars are outliers, i.e. they are more than farthest from 1.5 times the interquartile range of the first quartile and the third quartile.

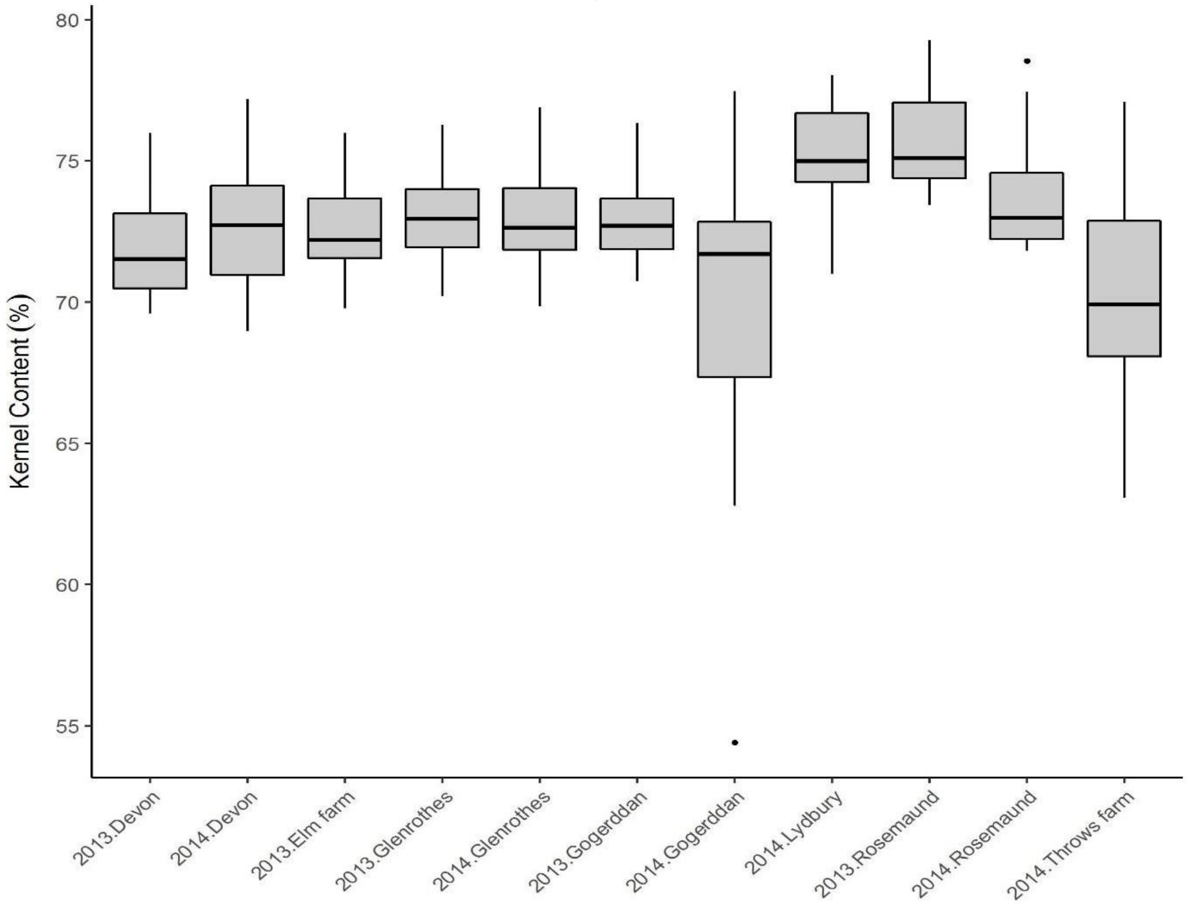


Figure 3.15. Box plot of k*ernel content (%) of the four winter oat varieties, Balado, Gerald, Mascani and Tardis, for each environment. The box plot* (Weisstein, 2018) *represents between first quartile (25 %) and the third quartile of the data (75 %), with the horizontal line inside the box indicating the median. The whiskers represent the data within 1.5 times the interquartile range of the first quartile and the third quartile. Data points represented by stars are outliers, i.e. they are more than farthest from 1.5 times the interquartile range of the first quartile and the third quartile.*

By locations (table 3.4, figure 3.15) the highest values for kernel content were obtained at Rosemaund 2013 and Lydbury 2014 (site 4 and 7 respectively) whereas the lowest values were obtained at Gogerddan and Throws farm in 2014. At the same time the range of values obtained for kernel content was widest in Gogerddan 2014 and Throws farm 2014. This is also reflected in the joint regression analysis presented in figure 3.16. Balado reached the lowest values (mean of 60.8%) at Gogerddan 2014 (site 6) which is far below the minimum required for the milling industry and end-user, and at Throws farm-2014 (site 11) with a mean of 65.4%. Gerald also had the lowest kernel contents at Gogerddan-2014.

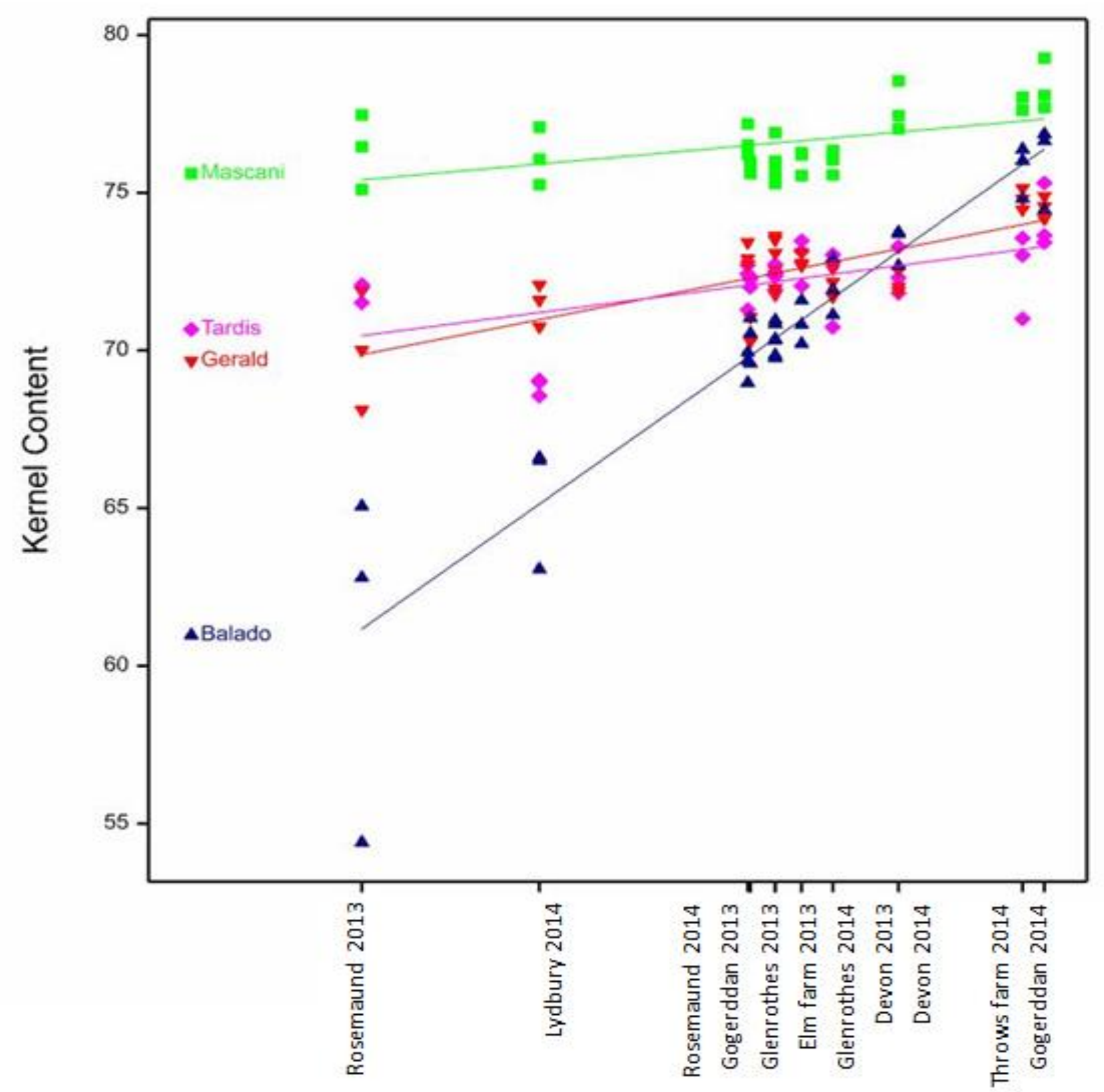


Figure 3.16. Joint regression plot (Finlay & Wilkinson, 1963), of four winter oat varieties kernel content values against sites for both harvest seasons 2013 and 2014.

Joint regression analysis (figure 3.16), showed Mascani as the most stable between environments, with a sensitivity value of 0.31 (table 3.6), also shown in the graph by the joint regression line and by the mean square deviation value (0.83), i.e. a more predictable response. The highest sensitivity to the environment was obtained for Balado indicating that it had the lowest stability and the least predictable response to the environments. These results are in accordance with the values on the ranking (numbers in brackets in the table), table 3.6, which also shows that Mascani is the cultivar with the highest superiority (0.00) and static stability (0.84), whilst Balado has the lowest values for these measures.

Table 3.6. *Average kernel content (%) overall seasons and sites, cultivar superiority, static stability and mean square deviation of the four winter oat varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Kernel Content (%) Varieties	Mean	Cultivar Superiority	Static Stability	Sensitivity	Mean square deviation
Balado	70.43	25.86(4)	19.01(4)	2.43(2)	2.74(4)
Gerald	72.46	9.09(2)	2.17(3)	0.68(4)	1.04(3)
Mascani	76.58	0.00(1)	0.84(1)	0.31(1)	0.83(1)
Tardis	72.19	10.29(3)	1.57(2)	0.45(3)	1.03(2)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*

3.3.4 Specific weight

Environment had a significant (p-value<0.001 two ways ANOVA) effect on specific weight (table 3.4) which ranged from 49.2 kg/hl at Rosemaund and Throws farm (site 10 and 11), both in 2014, to 53.7 kg/hl at Lydbury 2014 (site 7). A significant (p-value<0.001) difference was also found between varieties averaged over all environments. The specific weight of Mascani and Gerald was greater than Balado and Tardis for both harvest seasons. Balado had the lowest values in both harvest seasons, with a mean of 49.1 kg/hl in 2013 and 48.5 kg/hl in 2014 (figure 3.17). There was no significant difference between sensitivity values to the environment between varieties as shown by joint regression analysis (table 3.7, figure 3.18).

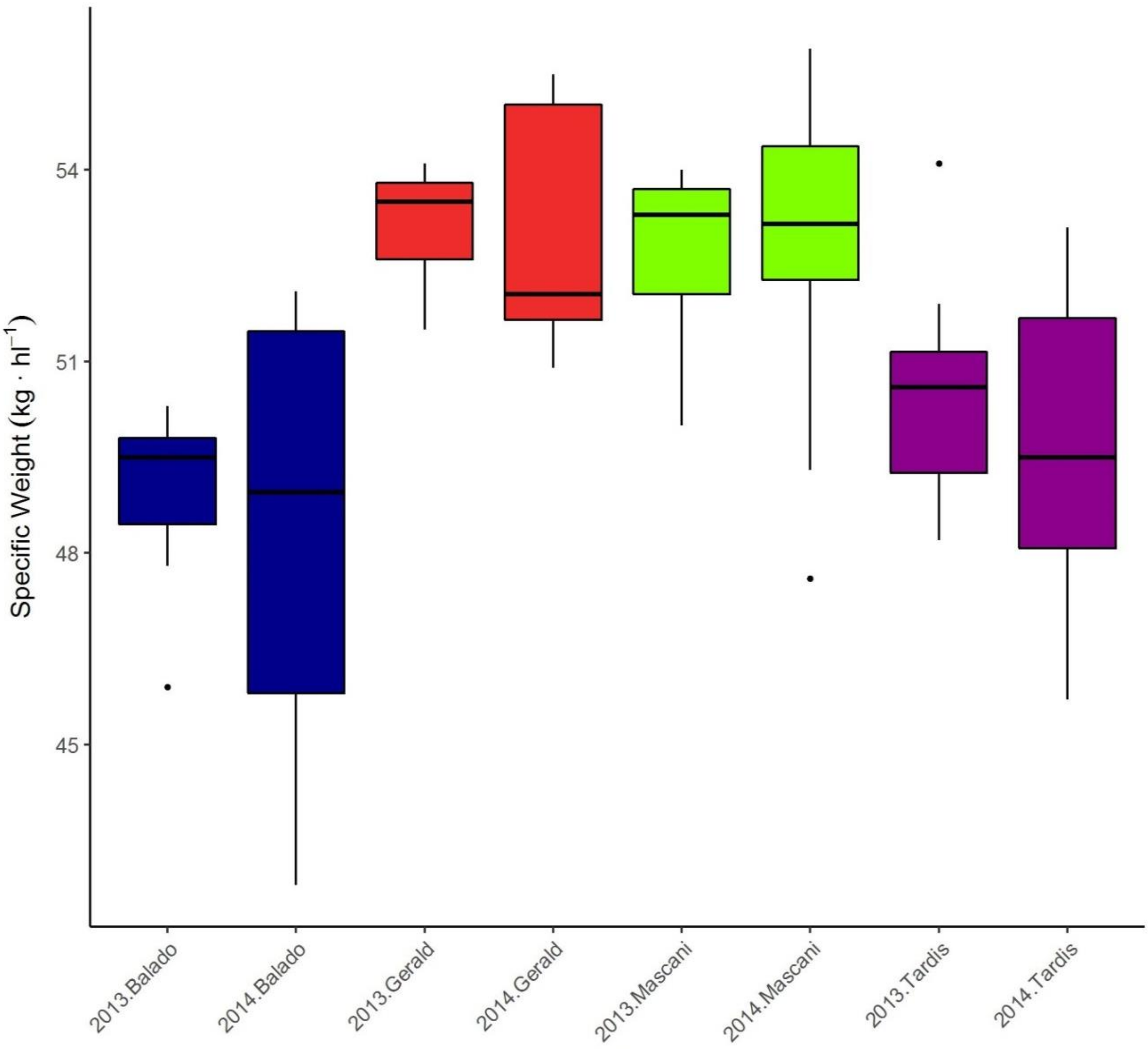


Figure 3.17. *Box plot of grain specific weight (kg/hl) of Balado, Gerald, Mascani and Tardis, average values for 2012-2013 and 2013-2014 harvest seasons. The box plot* (Weisstein, 2018) *represents between first quartile (25 %) and the third quartile of the data (75 %), with the horizontal line inside the box indicating the median. The whiskers represent the data within 1.5 times the interquartile range of the first quartile and the third quartile. Data points represented by stars are outliers, i.e. they are more than farthest from 1.5 times the interquartile range of the first quartile and the third quartile.*

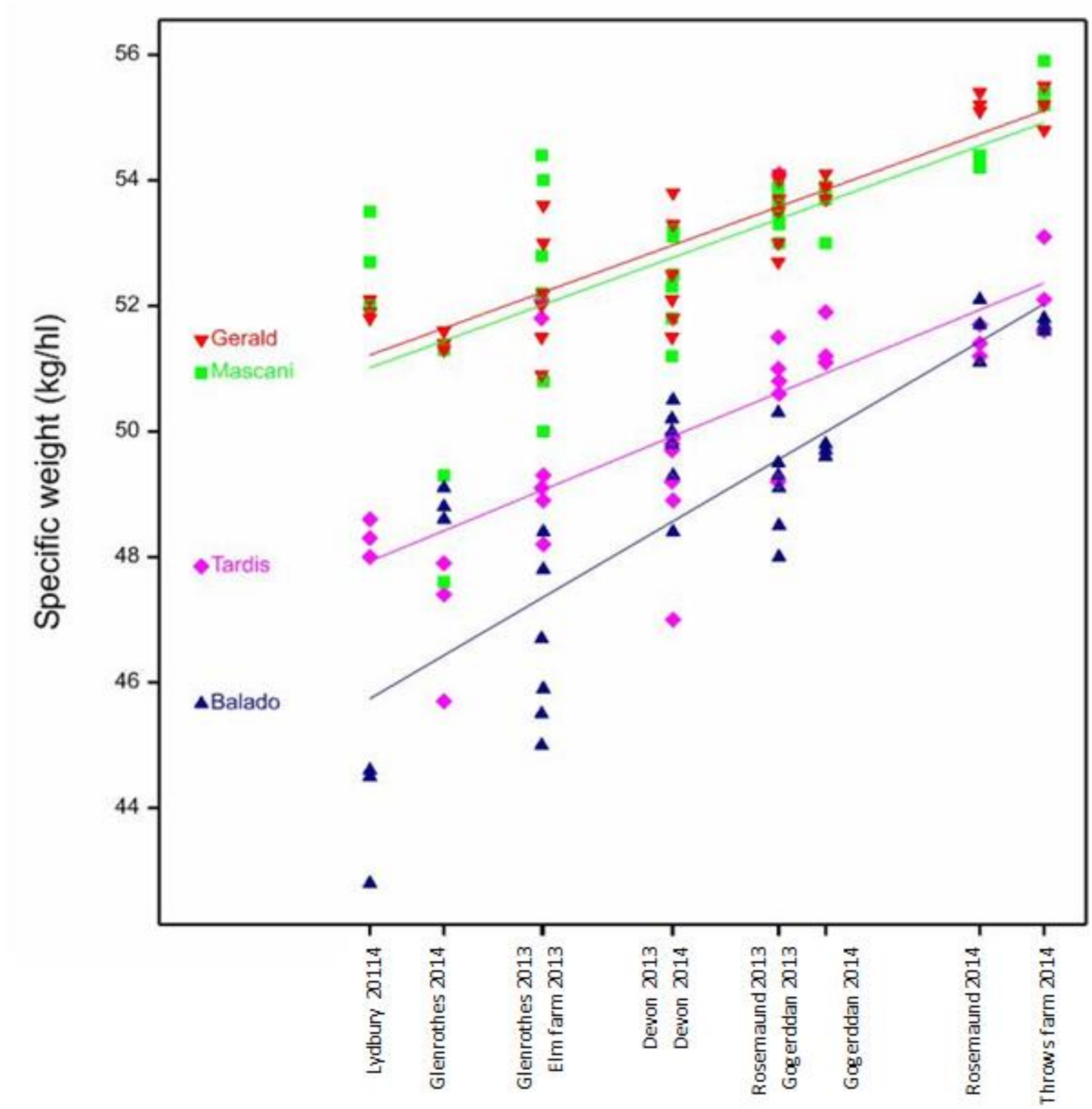


Figure 3.18. *Joint regression plot (Finlay & Wilkinson, 1963), of four winter oat varieties specific weight values against sites for both harvest seasons 2013 and 2014.*

Balado had the lowest specific weight in all environments. Gerald and Mascani had higher cultivar superiority, 0.18 and 0.34 respectively, and static stability, 1.81 and 2.53 (figure 3.18 and table 3.7). Balado and Tardis always had poorer values and ranked lower.

Table 3.7. *Average specific weight (t/hl) overall seasons and site, cultivar superiority, static stability and ranks of the four winter oat varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Specific Weight (t/hl) Varieties	Mean	Cultivar Superiority	Static Stability	Sensitivity	Mean square deviation
Balado	48.78	12.56(4)	5.47(4)	1.34(4)	1.87(4)
Gerald	53.10	0.18(1)	1.81(1)	0.83(1)	0.47(1)
Mascani	52.90	0.34(2)	2.52(2)	0.84(2)	1.67(2)
Tardis	50.07	5.98(3)	2.93(3)	0.95(3)	1.78(3)
Significance	*p-value*<0.001	*p-value*<0.001	*p-value*<0.001	*p-value*=0.158	*p-value*<0.001

3.3.5 Hullability

Regarding percentage hullability, genotypes were significantly different (p-value<0.001, two ways ANOVA) (table 3.8, figure 3.19). Mascani displayed very little variation in both harvest years (figure 3.19) remaining the highest at all sites with no value below 95% obtained for any environment. Balado, Gerald and Tardis, showed a wide range in results from 60% to 90% in both harvest years (figure 3.19). There were significant statistical differences (p-value<0.001) between sites, table 3.4 and the lowest values were obtained in the 2013 harvest season, regardless of the variety (figure 3.19). Rosemaund 2013 and Lydbury 2014 gave the highest values while Elm farm 2013 and Devon 2013 had the lowest hullability.

Genotype by site interaction were also statistically significant (p-value<0.001). When this is displayed in an environment centred analysis (figure 3.20) it can be seen that in all environments Mascani had a hullability higher than the mean and when compared with the rest of varieties. Gerald displayed hullability in all environments similar to the mean of those environments. Balado displayed a higher interaction with the environment for hullability and reached a maximum of 98.72%, at Rosemaund 2013 (site 4) and the lowest of 66.53%, at Devon 2013 (site 3). Gerald gave the best results at Lydbury 2014, 89.8% (site 7) and its lowest value at Devon 2013, 73.1% (site 3). Finally, Tardis achieved the highest value at Lydbury 2014, 83.8% (site 7) whilst the lowest was at Elm farm 2013, 62.9% (site 5).

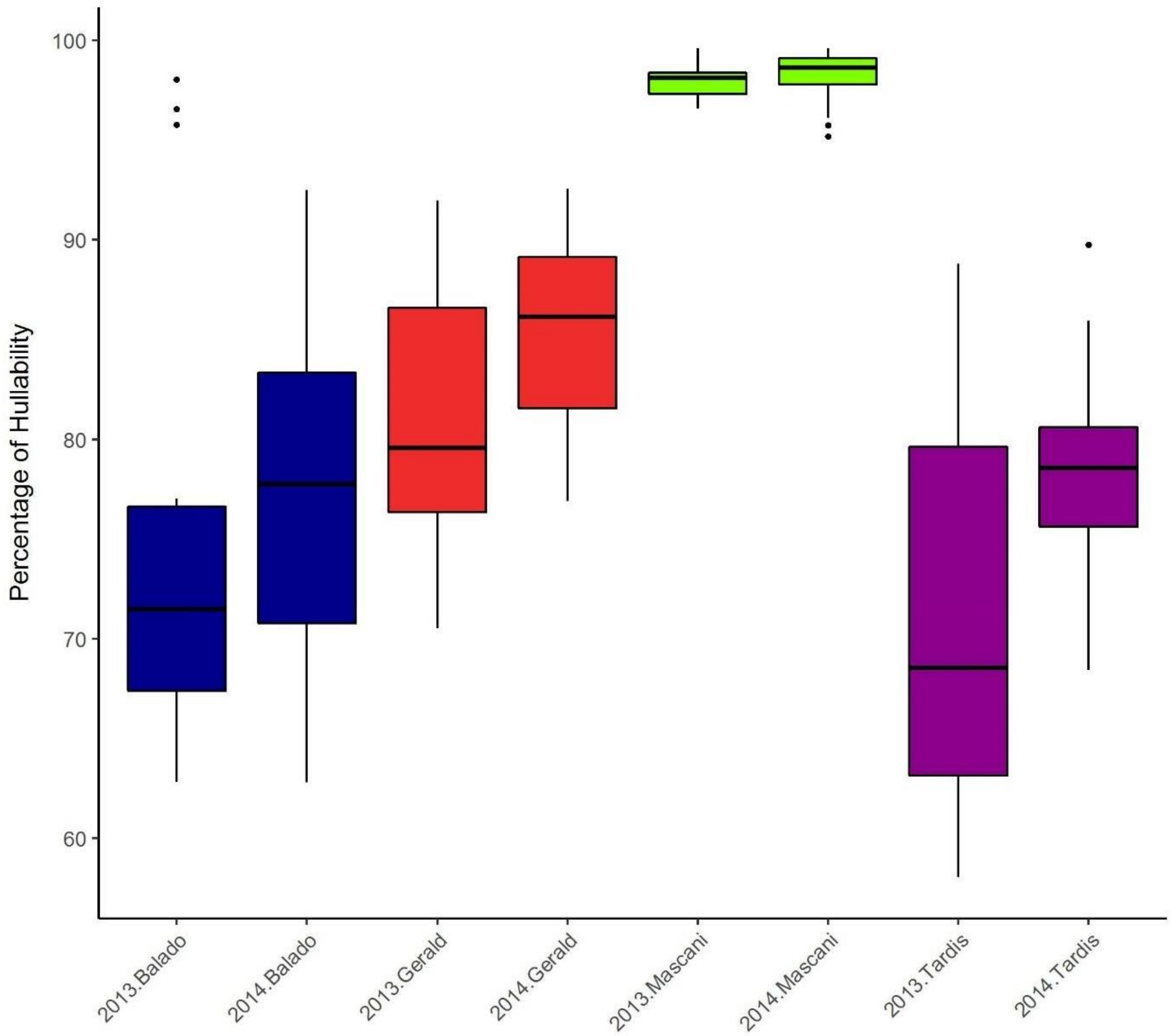


***Figure 3.19**. Box plot of hullability (%) values for each of the four winter oat varieties, i.e. Balado, Gerald, Mascani and Tardis, at each harvest season (2012/2013 and 2013/2014). The box plot* (Weisstein, 2018) *represents between first quartile (25 %) and the third quartile of the data (75 %), with the horizontal line inside the box indicating the median. The whiskers represent the data within 1.5 times the interquartile range of the first quartile and the third quartile. Data points represented by stars are outliers, i.e. they are more than farthest from 1.5 times the interquartile range of the first quartile and the third quartile.*

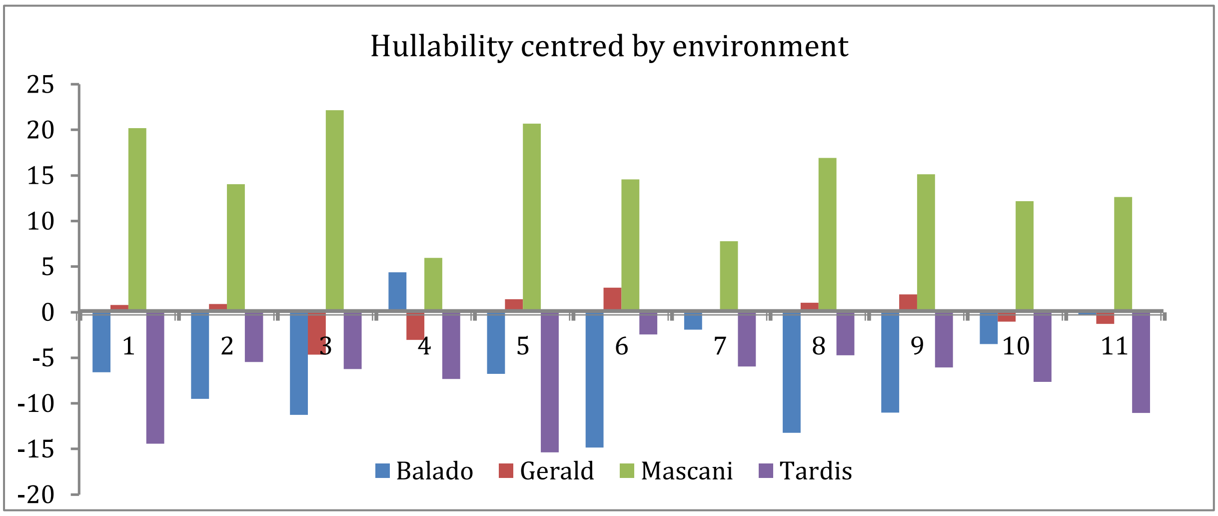


Figure 3.20. *Environment centred genotype hullability (%) by environment of the four winter oat varieties, Balado, Gerald, Mascani and Tardis, for 2012- 2013 and 2013-2014 harvest seasons*

Joint regression analysis (figure 3.21) showed no interaction with the environment for Mascani and it also had higher stability and superiority values (table 3.8). Mean square deviation values also showed that Mascani, with 1.13, gave a more predictable response to the environment. This, along with the lowest variance between environments, means that Mascani has the highest ranking. Balado, Gerald and Tardis showed more variable results. Sensitivity values for Balado, 1.68, resulted in a higher interaction with the environment (figure 3.21), lower values of stability performance and therefore, a more unpredictable behaviour. Tardis, although more stable than Balado in terms of sensitivity value, 1.26, gave a higher mean square deviation value (table 3.8), i.e. a more unpredictable response to the environment.

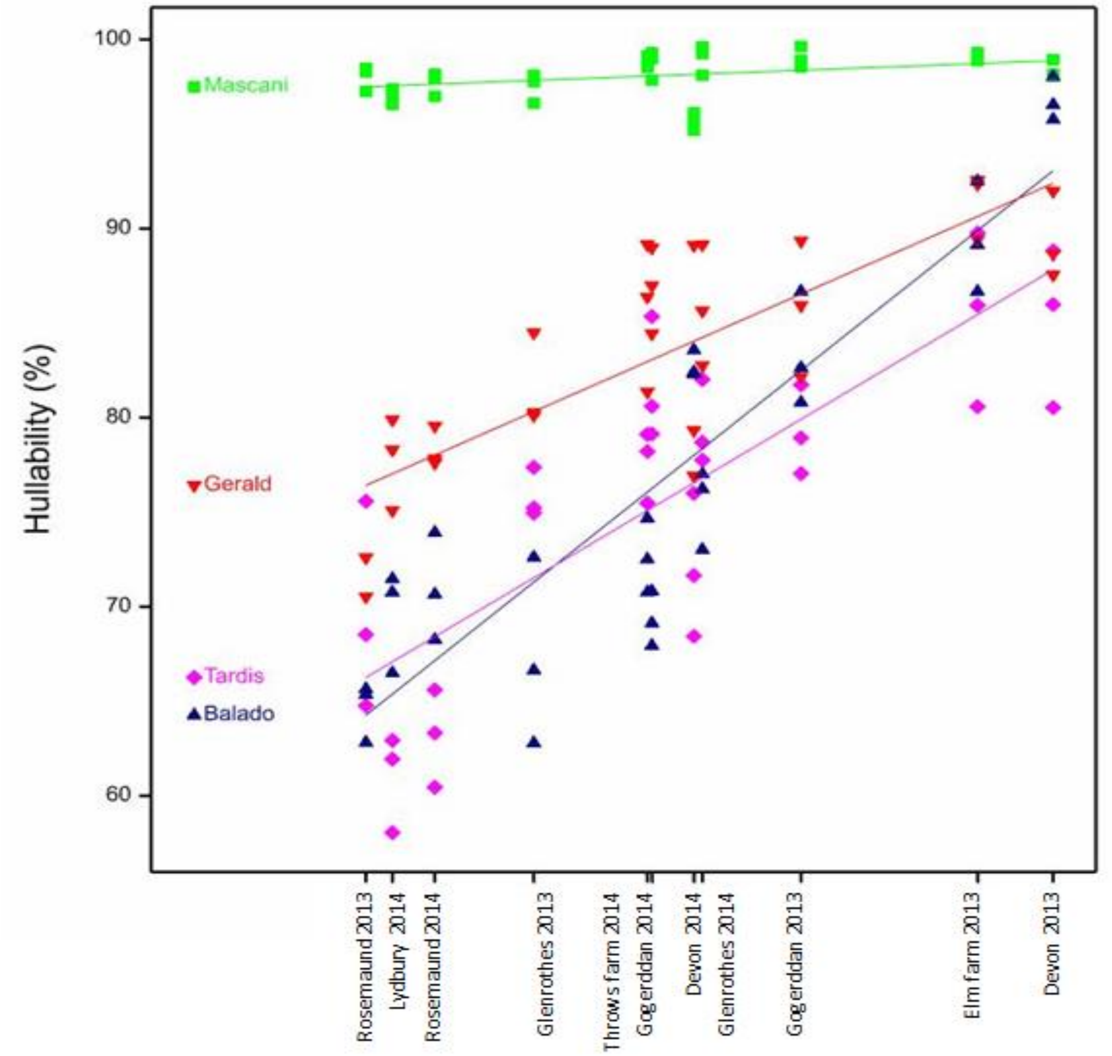


Figure 3.21. *Joint regression analysis of hullability (%) values for each of the four winter oat varieties at each of the eleven sites across UK at each harvest season (2012/2013 and 2013/2014).*

Table 3.8. *Average hullability (%) overall seasons and site, cultivar superiority, static stability and ranks of the four winter oat varieties over all environments. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Hullability (%)	Mean	Cultivar Superiority	Static Stability	Sensitivity	Mean square deviation
Varieties					
Balado	76.57	277.7(3)	103.77(4)	1.68(4)	19.54(3)
Gerald	83.24	123.3(2)	34.04(2)	0.93(2)	13.64(2)
Mascani	98.09	0.00(1)	1.12(1)	0.08(1)	1.13(1)
Tardis	75.47	282.2(4)	67.48(3)	1.26(3)	25.28(4)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*

3.3.6 Thousand grain weight

There was a significant (p-value<0.001, two way ANOVA) effect of environment on thousand grain weight (TGW) which ranged from 35.0 g (Throws farm 2014) to 47.8 g (Lydbury 2014) across the 11 environments (table 3.4) and a significant effect (p-value<0.001) of variety with the mean TGW of Mascani (45.1 g) significantly greater than Gerald (36.9 g) with Balado and Tardis intermediate (table 3.9). The results observed in 2013, for all varieties except Gerald, had a higher variance, (figure 3.22), in comparison with the 2014 harvest season.

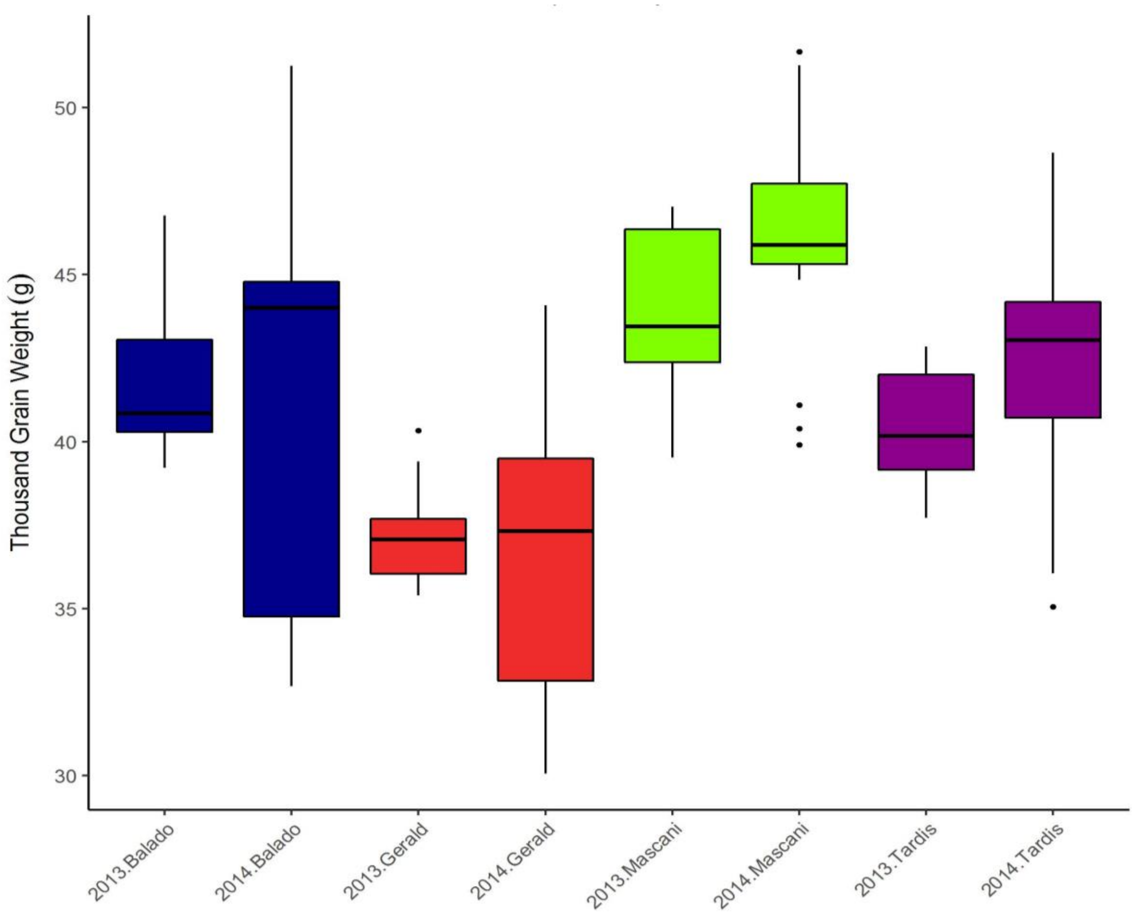


Figure 3.22. *Box plot for thousand Grain Weight (g) values of the four winter oat varieties, Balado, Gerald, Mascani and Tardis for 2012- 2013 and 2013-2014 harvest seasons. The box plot* (Weisstein, 2018) *represents between first quartile (25 %) and the third quartile of the data (75 %), with the horizontal line inside the box indicating the median. The whiskers represent the data within 1.5 times the interquartile range of the first quartile and the third quartile. Data points represented by stars are outliers, i.e. they are more than farthest from 1.5 times the interquartile range of the first quartile and the third quartile.*

There was also a significant (p-value<0.001) difference between varieties in their sensitivity to environment as shown by joint regression (table 3.9, figure 3.23). Mascani showed higher stability (8.97), superiority values (1.01) and with a sensitivity value of 0.718, and was the most stable variety, and therefore the first in the ranking (table 3.9). However, the mean square deviation of 4.09 for Mascani suggests that it does not display predictable response to the environment, while Tardis, second in ranking and with a higher sensitivity value, 0.89, gives the most predictable response.

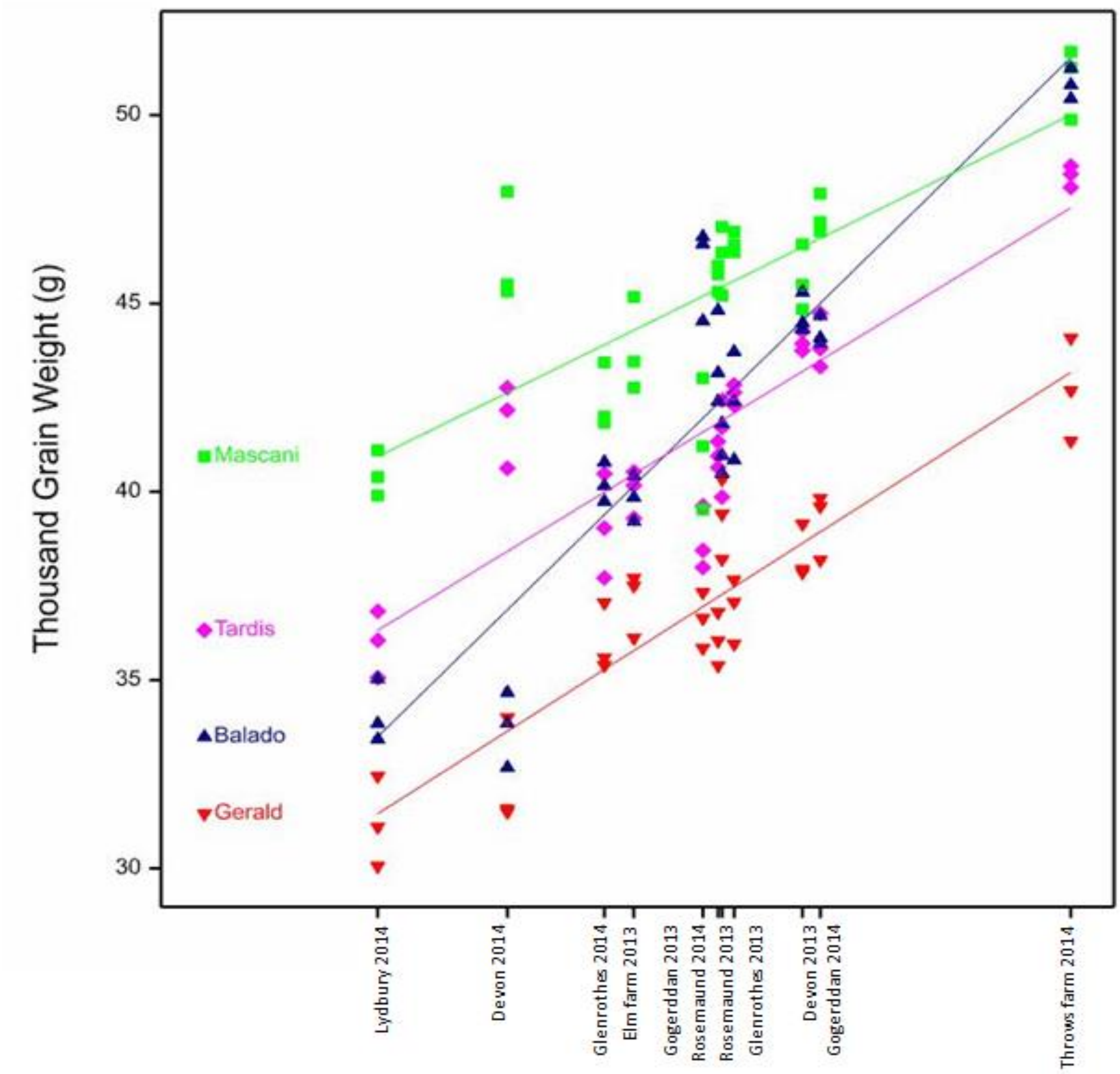


Figure 3.23. *Joint regression analysis of Thousand Grain Weight (g) from the four winter oat varieties and of the eleven sites across UK for 2012- 2013 and 2013-2014 harvest seasons.*

Table 3.9. *Average Thousand Grain Weight (g) overall seasons and site, cultivar superiority, static stability and ranks of the four winter oat varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Thousand Grain Weight (g) Varieties	Mean	Cultivar Superiority	Static Stability	Sensitivity	Mean square deviation
Balado	41.86	12.64(3)	24.73(4)	1.43(4)	3.59(3)
Gerald	36.89	39.73(4)	10.15(2)	0.93(3)	1.71(1)
Mascani	45.15	1.01(1)	8.97(1)	0.72(1)	4.09(4)
Tardis	41.53	9.13(2)	10.78(3)	0.89(2)	2.83(2)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*

Balado on the other hand, is the fourth in ranking, and therefore the least stable, (table 3.9). In environments where the mean TGW was high, Balado performed better (figure 3.23) with its regression line intersecting that for Tardis. However, its TGW was lower than Tardis in environments with a low mean TGW. Gerald, although more stable than Balado in terms of sensitivity value, 0.93 respectively 1.43, gave a lower mean square deviation value, which means that Gerald has a more unpredictable response to the environment.

Chemical Grain composition traits

Table 3.10. *Grain oil and protein content (%) and groat β-glucan content (%), average values ± s.e.m by site, i.e. eleven locations across the country and at each harvest season.*

Site	Site code	Year	Oil content	s.e.m	Protein content	s.e.m	B-Glucan content	s.e.m
Gogerddan	1	2013	6.96	0.06	12.12	0.07	4.15	0.04
Glenrothes	2	2013	6.91	0.06	11.60	0.06	4.18	0.04
Devon	3	2013	7.53	0.06	10.27	0.07	3.78	0.04
Rosemaund	4	2013	6.49	0.05	14.11	0.12	4.40	0.06
Elm farm	5	2013	7.32	0.05	11.33	0.05	3.87	0.05
Gogerddan	6	2014	7.91	0.07	8.92	0.03	3.33	0.07
Lydbury	7	2014	7.37	0.03	11.01	0.06	3.40	0.06
Glenrothes	8	2014	7.82	0.05	9.79	0.06	3.15	0.05
Devon	9	2014	7.33	0.04	9.06	0.06	3.53	0.07
Rosemaund	10	2014	7.40	0.05	9.66	0.03	3.40	0.06
Throws farm	11	2014	7.91	0.05	14.29	0.05	3.44	0.07
Overall average			7.36	0.16	11.11	0.27	3.69	0.09
Significance Genotype			*p-value<0.001*		*p-value<0.001*		*p-value<0.001*	
Significance Site			*p-value<0.001*		*p-value<0.001*		*p-value<0.001*	
Significance Interaction GxS			*p-value<0.001*		*p-value<0.001*		*p-value<0.001*	

3.3.7 Oil content

Significant differences (p-value<0.001, two way ANOVA) were obtained both between genotypes and between sites regarding grain oil content (%) (table 3.10). There were also, statistically significant interactions between the two factors. Average values by variety and of each year (figure 3.24), shows that Mascani has the lowest value among the varieties, reaching a minimum in 2013 of 6.4%, and an overall mean of 6.7% (table 3.10). On the other hand, Tardis gave the highest overall value, 7.7%, similar to Balado at 7.6%. Tardis was also more stable (figure 3.24), in terms of variance around the average, although with greater differences between seasons than Balado.

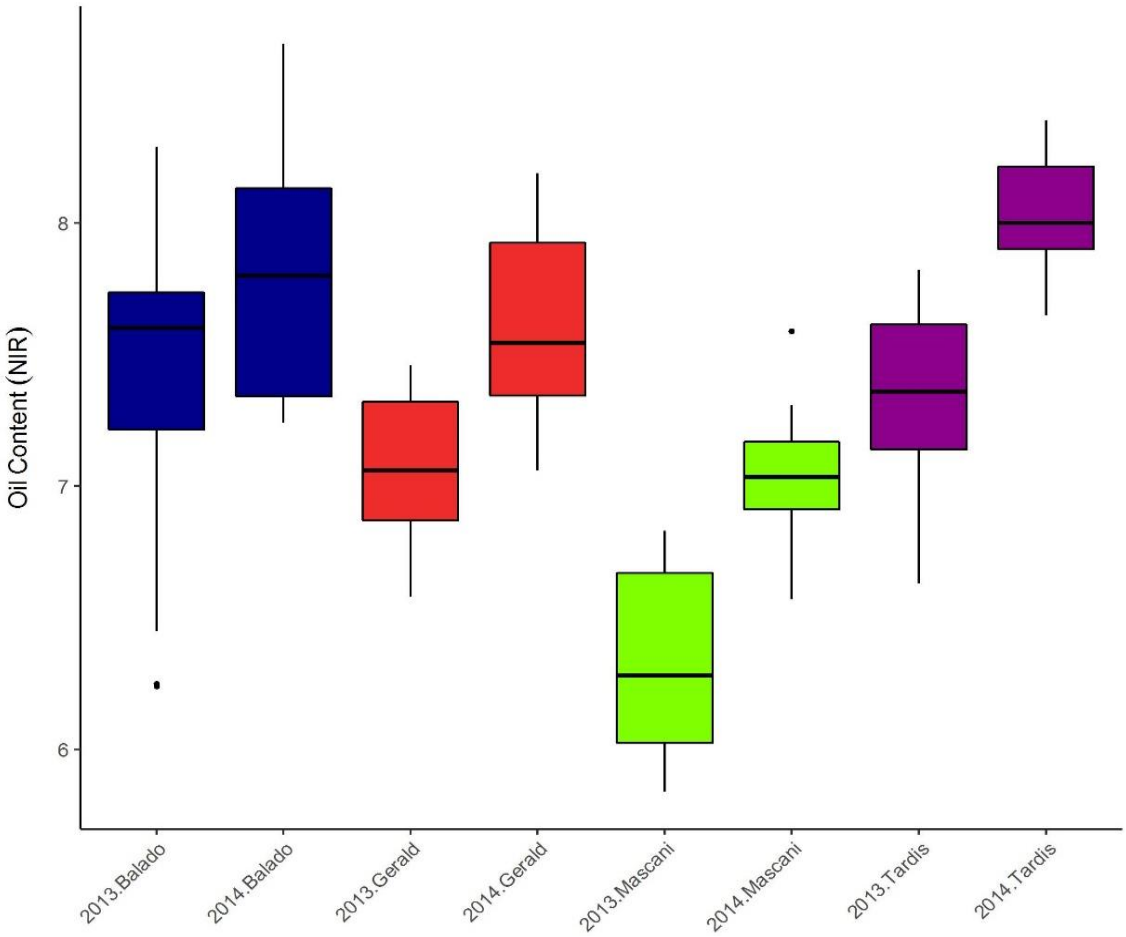


Figure 3.24. *Box plot of grain oil content (%) values of the four winter oat varieties, Balado, Gerald, Mascani and Tardis for 2012- 2013 and 2013-2014 harvest seasons. The box plot* (Weisstein, 2018) *represents between first quartile (25 %) and the third quartile of the data (75 %), with the horizontal line inside the box indicating the median. The whiskers represent the data within 1.5 times the interquartile range of the first quartile and the third quartile. Data points represented by stars are outliers, i.e. they are more than farthest from 1.5 times the interquartile range of the first quartile and the third quartile.*

By sites (table 3.10), the lowest oil content was from Rosemaund 2013 (site 3), with a value of 6.49% (table 3.10), while Gogerddan and Throws Farm, both in 2014, with 7.91% were highest in oil content. 2012/2013 harvest season had lower results in comparison with 2013/2014 harvest season by varieties and by sites.

Table 3.11. *Average oil content (g) overall seasons and site, cultivar superiority, static stability and ranks of the four winter oat varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Oil Content (%) Varieties	Mean	Cultivar Superiority	Static Stability	Sensitivity	Mean square deviation
Balado	7.5	0.05(2)	0.38(4)	1.25(4)	0.08(4)
Gerald	7.3	0.14(3)	0.16(1)	0.85(1)	0.03(1)
Mascani	6.7	0.65(4)	0.21(3)	0.95(3)	0.05(3)
Tardis	7.6	0.03(1)	0.20(2)	0.94(2)	0.04(2)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value=0.041*	*p-value<0.001*

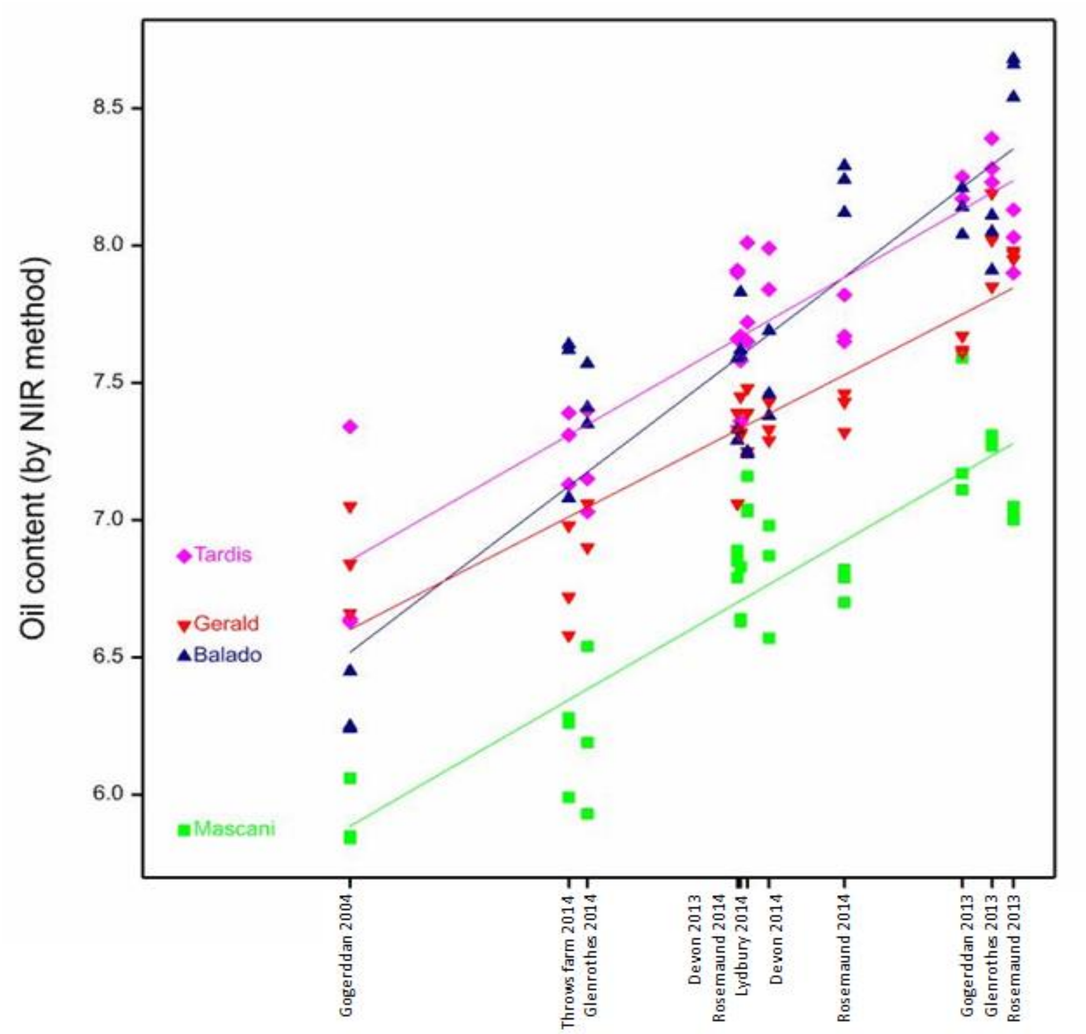


Figure 3.25. *Joint regression graph for grain oil content (%) values of the eleven sites across UK, for 2012- 2013 and 2013-2014 harvest seasons.*

There was also a significant (p-value<0.004) difference between varieties in their sensitivity to environment (figure 3.25). Balado (sensitivity 1.25) was the most sensitive to environment with Gerald (slope 0.85) least sensitive to environment, with Mascani and Tardis intermediate (table 3.11). Tardis with the highest average oil content, 7.6%, had the highest superiority value. Gerald with an average oil content of 7.3% had the highest static stability, meaning that it was the most stable between varieties. The mean square deviation values also show that Gerald had with the lowest value, indicating that it gave the most predictable response to the environment. Balado, on the other hand, having higher average oil content (7.5%) than Gerald (7.3%) and Mascani (6.7%), was lowest in static stability. It also had the highest mean square deviation so was the least stable and the most unpredictable of the four varieties.

3.3.8 Protein content

There were statistically significant differences amongst genotypes (p-value<0.001(two way ANOVA)) (table 3.12), sites (p-value<0.001(two ways ANOVA)) (table 3.12) and significant interactions between the two factors (p-value<0.001(two ways ANOVA)). The mean protein content (table 3.12 and figure 3.26) in 2013 was higher than 2014. Although Tardis had higher average protein content than Balado, 11.3% and 11.2% respectively in 2014, it was Balado overall seasons that had the highest protein content (table 3.12), followed by Tardis and Gerald. By sites (table 3.12), Rosemaund 2013 had the highest results for all varieties, with a minimum value of 12.5% from Gerald and a maximum value of 14.8% from Balado. Gogerddan 2014, with 8.94% was the lowest mean site protein content.

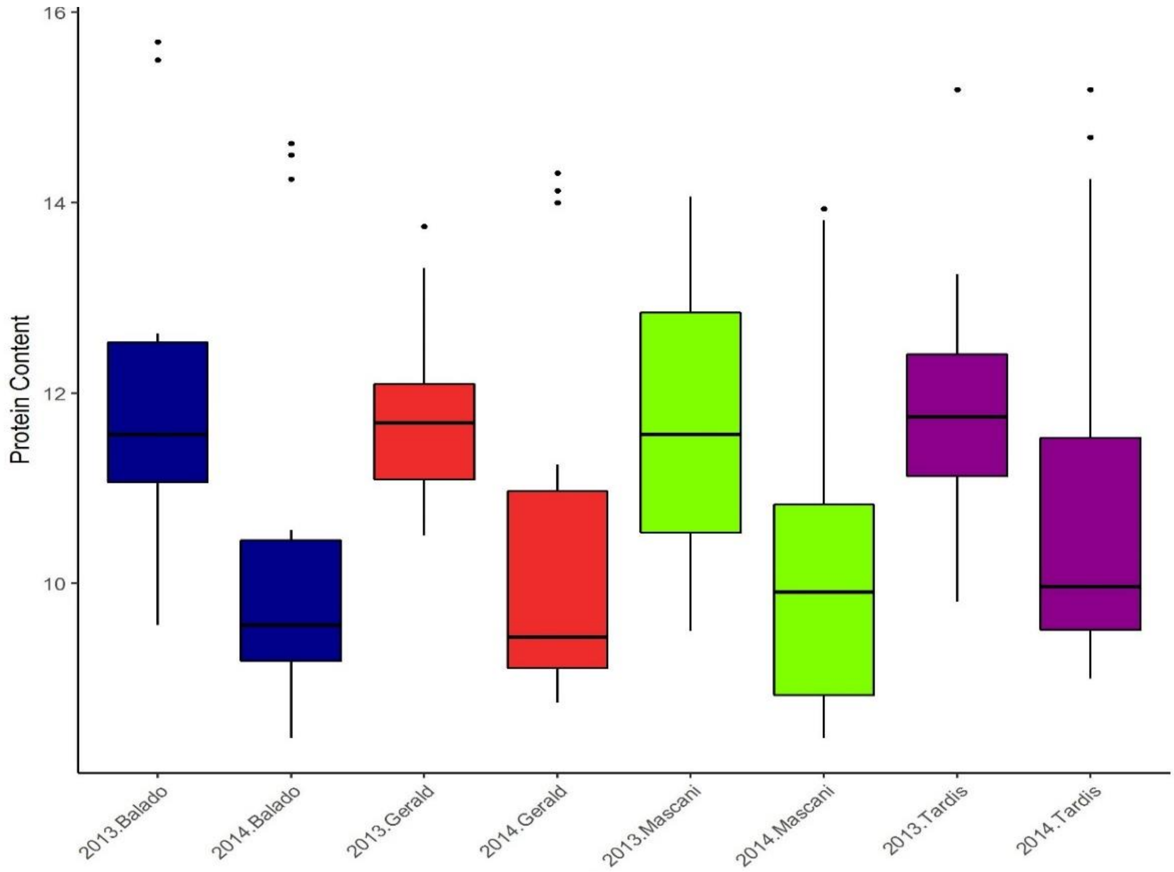


Figure 3.26. *Grain protein content (%) average value of the four winter oat varieties, Balado, Gerald, Mascani and Tardis for 2012- 2013 and 2013-2014 harvest seasons. The box plot* (Weisstein, 2018) *represents between first quartile (25 %) and the third quartile of the data (75 %), with the horizontal line inside the box indicating the median. The whiskers represent the data within 1.5 times the interquartile range of the first quartile and the third quartile. Data points represented by stars are outliers, i.e. they are more than farthest from 1.5 times the interquartile range of the first quartile and the third quartile.*

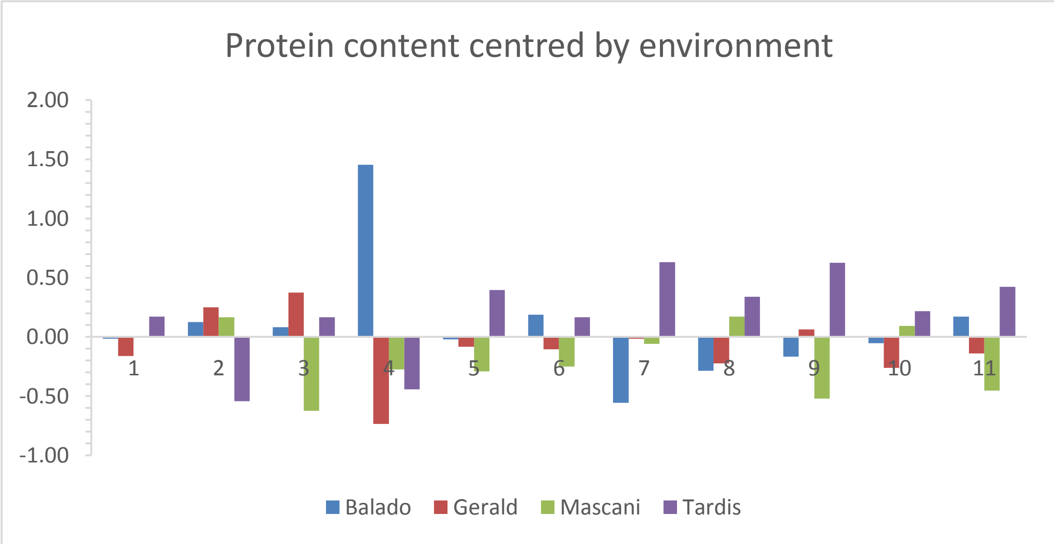


Figure 3.27. *Environment centred genotype grain protein content (%) by environment of the four winter oat varieties, Balado, Gerald, Mascani and Tardis, for 2012- 2013 and 2013-2014 harvest seasons*

The range of protein values as displayed in figure 3.27 reflects the sites that have the potential to discriminate between varieties´ performances, i.e. to investigate further in which sites a genotype protein values are better in comparison with the rest of the sites. Therefore, site 4, Rosemaund 2012/2013, site 9, Devon 2014, and site 10, Rosemaund 2014, showing a wider range of values suggest that these are the best environments to discriminate between the four varieties. The rest of the sites did not have visible differences in the performance of the different genotypes and therefore they are not useful to discriminate between genotypes.

Table 3.12. *Average protein content (g) overall seasons and site, cultivar superiority, static stability and ranks of the four winter oat varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Protein Content (%) Varieties	Mean	Cultivar Superiority	Static Stability	Sensitivity	Mean square deviations
Balado	11.6	0.13(1)	4.72(4)	1.16(4)	0.28(2)
Gerald	11.2	0.31(3)	3.03(2)	0.93(2)	0.12(1)
Mascani	11.2	0.33(4)	3.38(3)	0.99(3)	0.31(3)
Tardis	11.5	0.19(2)	3.02(1)	0.92(1)	0.36(4)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*

Joint regression analysis (table 3.12 and figure 3.28), showed statistical significance for sensitivity to the environment (p-value<0.001). The ranking showed Tardis as highest (table 3.12) in terms of static stability and sensitivity, despite having lower protein content. It however had the highest mean square deviation. Balado was highest in ranking in terms of cultivar superiority but also had the lowest stability and sensitivity across environments.

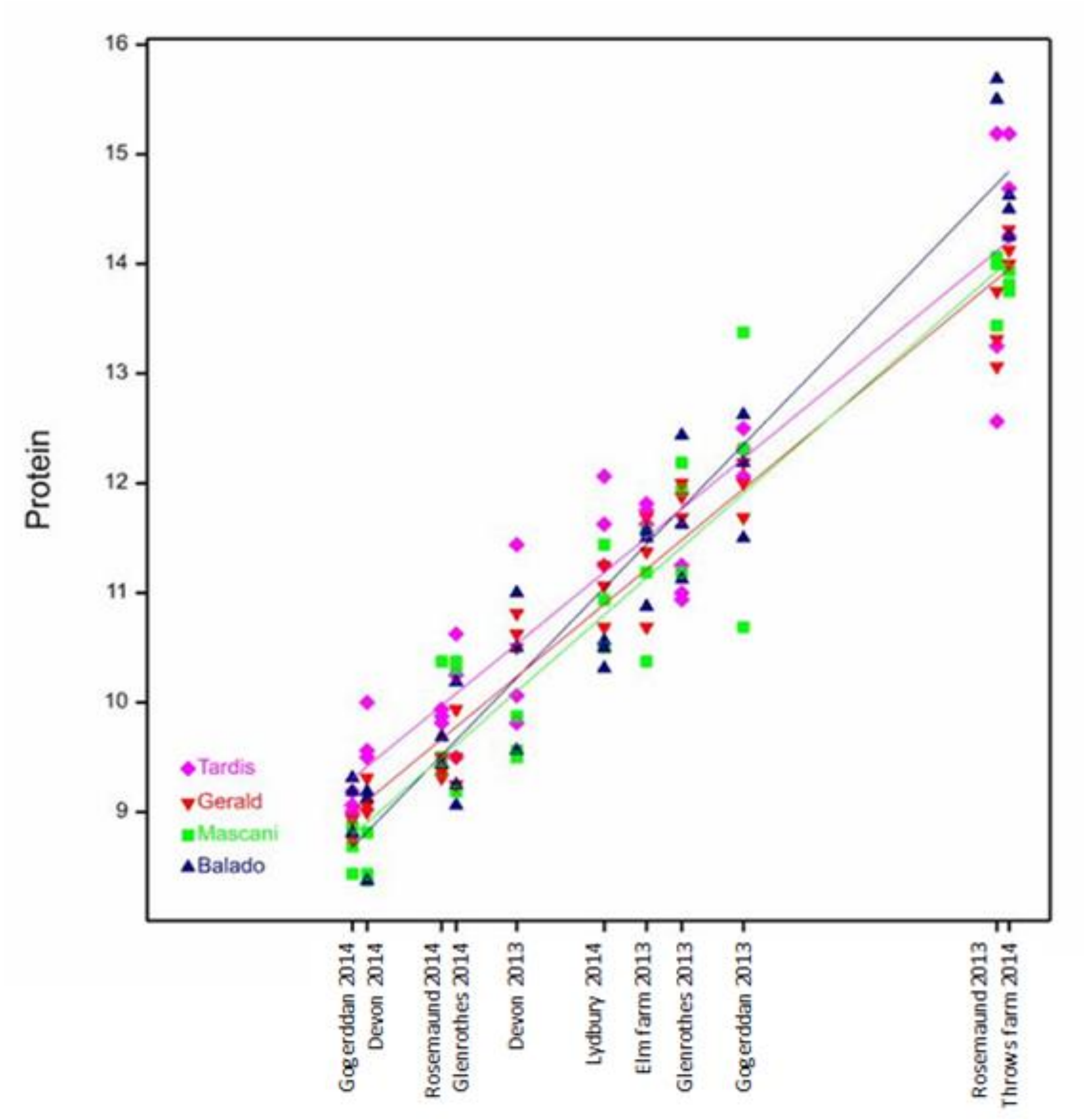


Figure 3.28. *Joint regression graph for grain protein content (%) values of the four winter oat varieties and the eleven sites across UK, for 2012- 2013 and 2013-2014 harvest seasons.*

3.3 9 β-Glucan content

There were statistically significant differences (p-value<0.001, two way ANOVA) between genotypes, sites and interaction between the two factors. Balado had the highest β-glucan content (table 3.13 and figure 3.29), in both harvest seasons with an overall average value of 4.6% (table 3.13), whilst Gerald had the lowest of 3.6%. Tardis, with an overall average value of 3.7%, showed a wider range of values than the rest of varieties. By sites (table 3.10), Rosemaund 2013 had the highest values (4.4%), whilst Devon in 2013 had the lowest value, 3.8%.

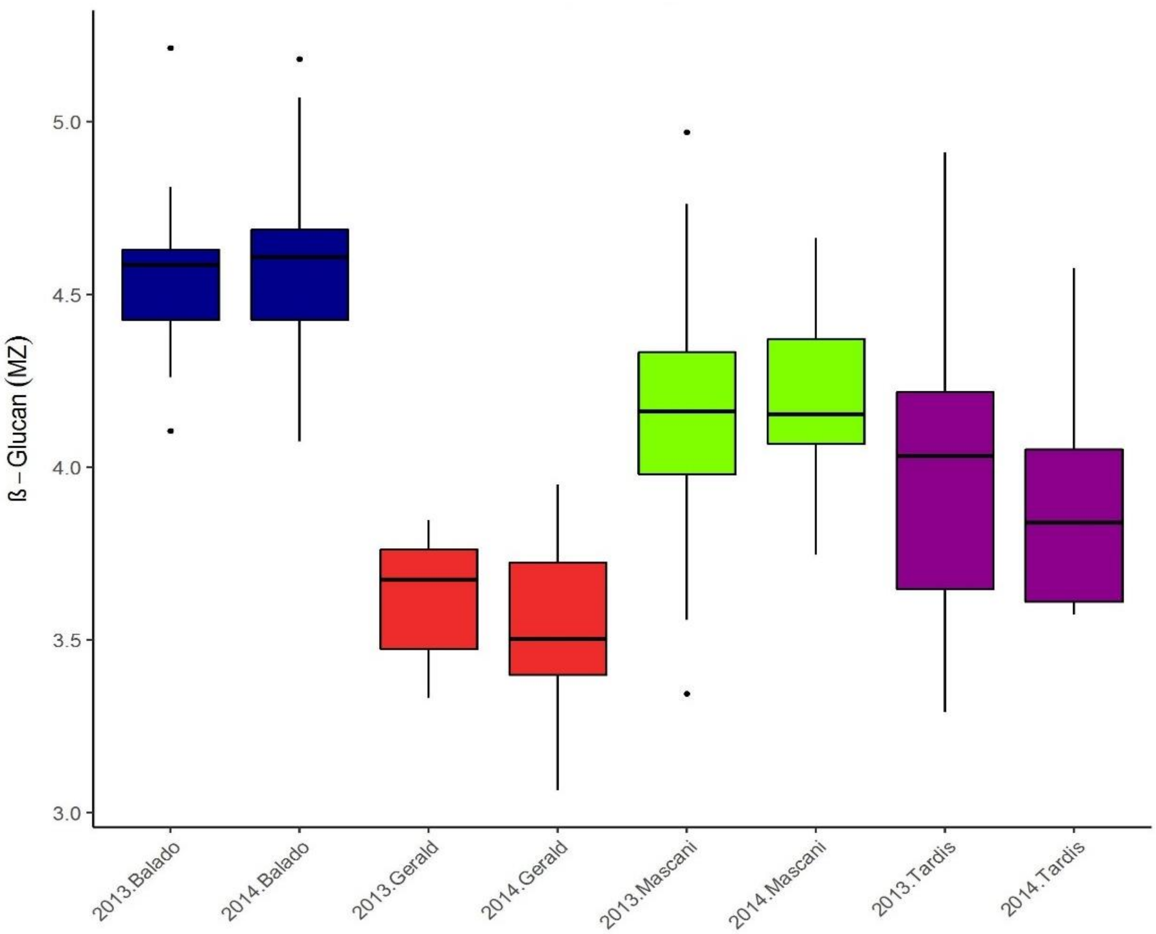


Figure 3.29. *Box plot of grain β-glucan content (%) values of the four winter oat varieties for 2012-2013 and 2013-2014 harvest seasons. The box plot* (Weisstein, 2018) *represents between first quartile (25 %) and the third quartile of the data (75 %), with the horizontal line inside the box indicating the median. The whiskers represent the data within 1.5 times the interquartile range of the first quartile and the third quartile. Data points represented by stars are outliers, i.e. they are more than farthest from 1.5 times the interquartile range of the first quartile and the third quartile.*

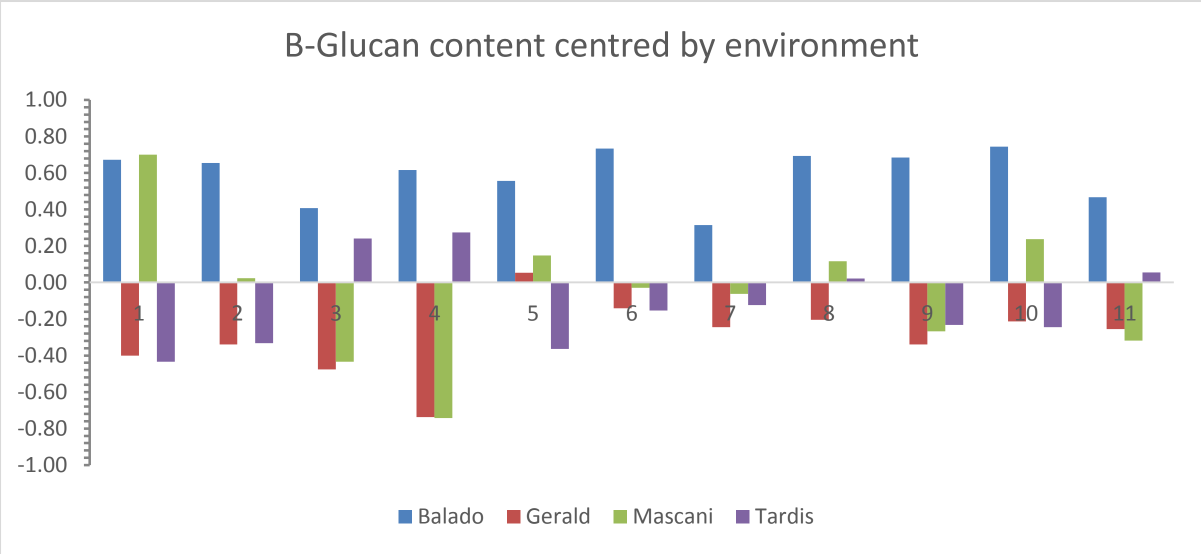


Figure 3.30. *Environment centred genotype grain β-glucan content (%) by environment of the four winter oat varieties, Balado, Gerald, Mascani and Tardis, for 2012- 2013 and 2013-2014 harvest seasons.*

The effect of environment on β-glucan (figure 3.30), indicates that at almost all sites, Balado had the highest values. This analysis also indicates the sites that would be more interesting to discriminate between varieties´ performances. However, all sites showed similar results, with no visible differences in the performance of the different genotypes and therefore no site was identified that was useful to discriminate between genotypes.

Joint regression analysis showed statistically significant sensitivity values (p-value<0.05) (table 3.13 and figure 3.31), indicating that there is variation in genotype behaviour with changing environments. Cultivar superiority, table 3.13 and figure 3.31, shows Balado with the highest β-glucan content, 4.6%. The most stable across environments is Gerald with 0.03 static stability and the lower mean square deviation, 0.04, but it had also the lowest mean value (table 3.13). The variety with the highest sensitivity (1.64) was Tardis.

Table 3.13. *Average β-glucan content (%), cultivar superiority, static stability and mean square deviation of the four winter varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

β-Glucan Content (%) Varieties	Mean	Cultivar Superiority	Static Stability	Sensitivity	Mean square deviation
Balado	4.3	3.6e-6(1)	0.04(2)	0.64(2)	0.06(3)
Gerald	3.6	0.56(4)	0.03(1)	0.38(1)	0.04(1)
Mascani	3.9	0.13(2)	0.08(3)	1.20(3)	0.07(4)
Tardis	3.7	0.26(3)	0.11(4)	1.64(4)	0.05(2)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value=0.003*	*p-value<0.001*

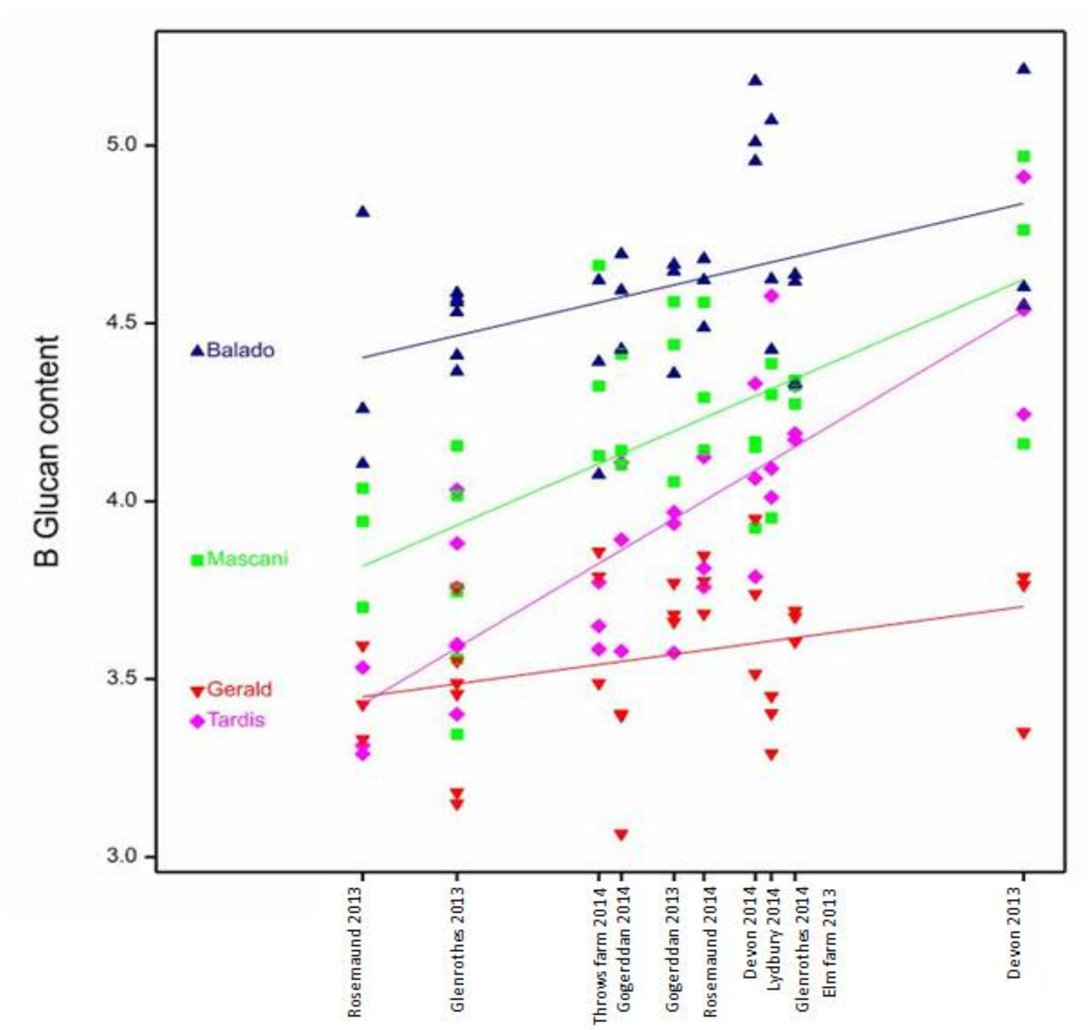


Figure 3.31. *Joint regression graph for grain β-glucan content (%) as measured using the megazime (MZ) method for the four winter oat varieties and the eleven sites across UK, for 2012-2013 and 2013-2014 harvest seasons.*

3.3.10 Shape grain analysis. Individual grains

MARVIN Image analysis was conducted on the grain prior to de-hulling and on the de-hulled groats of the same sample used for kernel content and hullability determination. From the results obtained, a mean value for a range of grain and groat dimensions were determined for each sample, i.e. width, length, area and grain and groat ratio, and shape descriptors, i.e. circularity, compactness. Two way ANOVA showed statistical significance differences between genotypes (p-value<0.001), environments (p-value<0.001) and significant interactions between them (p-value<0.001), (table 3.14 and 3.15), for all grain size and shape traits under study.

Gerald had the lowest mean grain and groat area values (table 3.15 and 3.16.a and 3.16.b), but the lowest mean square deviation in both cases, meaning that it was the more stable in grain and groat area. The variety with the highest sensitivity to the environment and the highest static stability grain area value however, was Balado (table 3.16). The highest mean grain area was found in Balado at Rosemaund in 2013 with a value of 30 mm^2 whereas the lowest value was obtained for Gerald at Throws Farm 2014 (22 mm^2). In terms of groat area, Mascani displayed the highest mean value (table 3.16.b) with also the best cultivar superiority and static stability performance. Interestingly, Gerald in terms of groat area had the lowest cultivar superiority value.

Table 3.14. *Mean grain and groat width (mm), length (mm) and area (mm^2) of each site and harvest seasons, 2012/2013 and 2013/2014, of the four winter varieties.*

Site	Site code	Year	Area mm^2	s.e.m	Width mm	s.e.m	Length mm	s.e.m	Grain Ratio	s.e.m	Area Groat mm^2	s.e.m	Width Groat mm	s.e.m	Length Groat mm	s.e.m	Groat Ratio	s.e.m
Gogerddan	1	2013	28.5	1.7	3.1	0.0	12.8	0.8	0.25	0.3	15.5	1.0	2.6	0.0	7.1	0.4	0.36	0.2
Glenrothes	2	2013	26.7	1.3	3.1	0.0	11.9	0.6	0.26	0.2	15.1	0.8	2.5	0.0	7.1	0.3	0.36	0.1
Devon	3	2013	27.3	1.3	3.1	0.0	12.5	0.7	0.25	0.2	14.3	0.9	2.5	0.0	6.8	0.3	0.36	0.1
Rosemaund	4	2013	28.0	1.5	3.1	0.1	12.6	0.5	0.24	0.2	15.5	1.5	2.5	0.1	7.3	0.5	0.34	0.1
Elm farm	5	2013	27.2	1.6	3.1	0.0	12.2	0.7	0.26	0.2	15.3	1.1	2.6	0.1	7.1	0.4	0.37	0.1
Gogerddan	6	2014	24.8	1.8	3.1	0.1	10.7	0.5	0.29	0.2	13.8	1.5	2.5	0.1	6.6	0.4	0.38	0.1
Lydbury	7	2014	26.6	1.5	3.4	0.1	10.7	0.5	0.31	0.1	16.3	1.0	2.7	0.1	7.1	0.3	0.38	0.1
Glenrothes	8	2014	25.8	1.5	3.2	0.0	10.8	0.6	0.30	0.2	14.9	0.9	2.6	0.0	6.8	0.3	0.38	0.1
Devon	9	2014	26.4	1.4	3.2	0.0	11.0	0.6	0.29	0.2	15.1	0.9	2.6	0.0	6.8	0.3	0.39	0.1
Rosemaund	1	2014	25.6	1.5	3.1	0.1	11.1	0.6	0.28	0.2	14.7	1.2	2.5	0.1	6.9	0.4	0.37	0.1
Throws farm	1	2014	23.9	1.4	2.9	0.1	10.7	0.5	0.28	0.1	13.7	1.1	2.4	0.1	6.8	0.3	0.36	0.1
Overall Mean			26.46	0.59	3.09	0.01	11.54	0.26	0.27	0.01	14.94	0.34	2.55	0.06	6.95	0.15	0.37	0.01
Significance Genotype			*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*	
Significance Site			*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*	
Significance Interaction			*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*	

Table 3.15. *Grain and groat size and shape, i.e. area (mm²), length (mm), width (mm), and ratio average values ± s.e.m. by variety, at each location and harvest season*

Selection	Site	Site code	Year	Area	s.e.m	Width	s.e.m	Length	s.e.m	Grain ratio	s.e.m	Area Groat	s.e.m	Width Groat	s.e.m	Length Groat	s.e.m	Groat ratio	s.e.m
Balado	Gogerddan	1	2013	28.5	0.6	3.1	0.0	12.9	0.5	0.24	0.0	15.4	0.1	2.5	0.0	7.2	0.1	0.35	0.0
	Glenrothes	2	2013	27.7	0.3	3.0	0.0	12.5	0.0	0.24	0.0	15.0	0.4	2.5	0.0	7.2	0.1	0.34	0.0
	Devon	3	2013	28.5	0.5	3.1	0.0	13.2	0.2	0.23	0.0	14.1	0.1	2.4	0.0	6.9	0.0	0.35	0.0
	Rosemaund	4	2013	30.0	0.2	3.2	0.0	13.0	0.1	0.24	0.0	17.5	0.3	2.6	0.0	8.0	0.0	0.32	0.0
	Elm farm	5	2013	28.0	0.5	3.1	0.0	12.6	0.1	0.25	0.0	15.4	0.2	2.5	0.0	7.3	0.1	0.34	0.0
	Gogerddan	6	2014	24.4	0.4	2.9	0.0	10.9	0.2	0.27	0.0	12.5	0.2	2.3	0.0	6.5	0.1	0.36	0.0
	Lydbury	7	2014	28.0	0.3	3.5	0.0	11.0	0.2	0.31	0.0	17.0	0.1	2.8	0.0	7.4	0.0	0.38	0.0
	Glenrothes	8	2014	27.3	0.2	3.2	0.0	11.4	0.1	0.28	0.0	15.3	0.2	2.6	0.0	7.0	0.0	0.37	0.0
	Devon	9	2014	27.5	0.6	3.2	0.0	11.6	0.4	0.28	0.0	15.6	0.3	2.6	0.0	7.0	0.1	0.37	0.0
	Rosemaund	10	2014	26.5	0.3	3.1	0.0	11.3	0.1	0.28	0.0	15.1	0.3	2.5	0.0	7.1	0.0	0.36	0.0
	Throws farm	11	2014	23.8	0.1	2.9	0.0	10.8	0.0	0.27	0.0	13.6	0.2	2.3	0.0	6.9	0.1	0.34	0.0
	Overall Mean			27.5	1.8	3.1	0.2	12.0	0.9	0.3	0.0	15.4	1.4	2.5	0.1	7.2	0.4	0.3	0.0
Gerald	Gogerddan	1	2013	26.2	0.7	3.1	0.0	11.6	0.2	0.27	0.0	14.2	0.4	2.6	0.0	6.6	0.1	0.39	0.0
	Glenrothes	2	2013	24.8	0.2	3.1	0.0	10.9	0.0	0.28	0.0	14.0	0.3	2.5	0.0	6.6	0.1	0.38	0.0
	Devon	3	2013	25.4	0.1	3.0	0.0	11.6	0.0	0.26	0.0	13.3	0.3	2.5	0.0	6.4	0.1	0.39	0.0
	Rosemaund	4	2013	26.2	0.1	3.0	0.0	11.8	0.2	0.26	0.0	13.9	0.2	2.4	0.0	6.7	0.1	0.36	0.0
	Elm farm	5	2013	24.6	0.4	3.1	0.0	11.0	0.1	0.28	0.0	13.7	0.1	2.6	0.0	6.4	0.0	0.40	0.0
	Gogerddan	6	2014	22.4	0.8	2.9	0.0	10.0	0.3	0.29	0.0	12.5	0.5	2.5	0.0	6.0	0.2	0.41	0.0
	Lydbury	7	2014	24.3	0.6	3.2	0.0	10.0	0.2	0.32	0.2	14.6	0.5	2.7	0.1	6.6	0.1	0.40	0.0
	Glenrothes	8	2014	23.7	0.3	3.1	0.0	10.0	0.1	0.31	0.0	13.4	0.4	2.6	0.0	6.3	0.1	0.41	0.0
	Devon	9	2014	24.2	0.2	3.1	0.0	10.2	0.1	0.31	0.0	14.3	1.0	2.6	0.0	6.5	0.4	0.40	0.0

	Rosemaund	10	2014	23.2	0.5	3.0	0.0	10.3	0.2	0.29	0.0	12.9	0.3	2.5	0.0	6.2	0.1	0.40	0.0
	Throws farm	11	2014	22.0	0.4	2.9	0.0	9.9	0.0	0.29	0.0	12.5	0.2	2.4	0.0	6.2	0.0	0.38	0.0
	Overall Mean			24.4	1.4	3.1	0.1	10.8	0.7	0.3	0.0	13.6	0.7	2.5	0.1	6.5	0.2	0.4	0.0
Mascani	Gogerddan	1	2013	29.8	1.4	3.2	0.0	13.2	0.6	0.24	0.0	16.9	0.3	2.6	0.0	7.5	0.2	0.35	0.0
	Glenrothes	2	2013	27.7	0.7	3.1	0.0	12.0	0.3	0.26	0.0	16.1	0.3	2.6	0.0	7.3	0.0	0.35	0.0
	Devon	3	2013	27.6	0.7	3.1	0.0	12.3	0.2	0.25	0.0	15.5	0.2	2.5	0.0	7.1	0.1	0.36	0.0
	Rosemaund	4	2013	28.2	1.0	3.1	0.0	12.6	0.4	0.24	0.0	15.7	0.4	2.4	0.1	7.5	0.0	0.32	0.0
	Elm farm	5	2013	28.2	0.2	3.2	0.0	12.4	0.1	0.26	0.0	16.6	0.1	2.7	0.0	7.3	0.0	0.36	0.0
	Gogerddan	6	2014	26.1	0.5	3.2	0.0	10.7	0.2	0.30	0.0	15.8	0.3	2.7	0.0	7.0	0.1	0.38	0.0
	Lydbury	7	2014	26.7	0.5	3.3	0.1	10.6	0.1	0.31	0.0	16.9	0.2	2.8	0.0	7.2	0.1	0.39	0.0
	Glenrothes	8	2014	25.7	0.7	3.2	0.0	10.5	0.3	0.31	0.0	15.8	0.4	2.7	0.0	6.9	0.2	0.39	0.0
	Devon	9	2014	26.5	0.2	3.3	0.0	10.6	0.1	0.31	0.0	15.4	1.4	2.7	0.1	6.8	0.4	0.37	0.0
	Rosemaund	10	2014	26.1	0.2	3.2	0.0	10.9	0.1	0.29	0.0	16.0	0.2	2.6	0.0	7.1	0.0	0.36	0.0
	Throws farm	11	2014	25.0	0.4	3.0	0.0	10.8	0.1	0.28	0.0	15.4	0.1	2.6	0.0	7.1	0.0	0.36	0.0
	Overall Mean			27.2	1.4	3.2	0.1	11.6	1.0	0.3	0.0	16.0	0.5	2.6	0.1	7.2	0.2	0.4	0.0
Tardis	Gogerddan	1	2013	29.4	0.6	3.1	0.0	13.4	0.2	0.23	0.0	15.4	0.5	2.6	0.0	7.2	0.1	0.36	0.0
	Glenrothes	2	2013	26.6	0.1	3.1	0.0	12.1	0.1	0.25	0.0	15.1	0.5	2.5	0.0	7.1	0.1	0.35	0.0
	Devon	3	2013	27.8	0.4	3.0	0.0	13.1	0.2	0.23	0.0	14.2	0.3	2.5	0.0	6.8	0.1	0.36	0.0
	Rosemaund	4	2013	27.7	0.4	3.0	0.0	12.9	0.3	0.23	0.0	14.7	1.0	2.4	0.1	7.2	0.3	0.33	0.0
	Elm farm	5	2013	27.9	0.1	3.1	0.0	12.7	0.1	0.25	0.0	15.4	0.2	2.6	0.0	7.2	0.1	0.36	0.0
	Gogerddan	6	2014	26.5	0.3	3.2	0.0	11.3	0.2	0.28	0.0	14.6	0.5	2.5	0.1	6.9	0.1	0.36	0.0
	Lydbury	7	2014	27.5	0.1	3.3	0.0	11.1	0.1	0.30	0.0	16.7	0.1	2.7	0.0	7.4	0.0	0.37	0.0
	Glenrothes	8	2014	26.6	0.3	3.2	0.0	11.2	0.1	0.29	0.0	15.2	0.1	2.6	0.0	7.0	0.0	0.37	0.0
	Devon	9	2014	27.3	0.1	3.2	0.0	11.4	0.1	0.28	0.0	15.1	0.3	2.6	0.0	6.9	0.0	0.38	0.0
	Rosemaund	10	2014	26.6	0.8	3.1	0.0	11.8	0.4	0.26	0.0	14.8	0.2	2.5	0.0	7.0	0.0	0.36	0.0
	Throws farm	11	2014	25.0	0.4	3.0	0.0	11.1	0.1	0.27	0.0	13.9	0.1	2.4	0.0	6.9	0.0	0.35	0.0
	Overall Mean			27.2	1.1	3.1	0.1	12.1	0.3	0.3	0.0	15.0	0.7	2.5	0.1	7.1	0.2	0.4	0.0

Table 3.16.a. *Mean grain area (mm^2), cultivar superiority, static stability and mean square deviation values of the four winter varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Area (mm^2)	*Mean*	*Cultivar Superiority*	*Static Stability*	*Sensitivity*	*Mean Square Deviation*
Varieties					
Balado	27.43	0.37(2)	3.00(4)	1.31(4)	0.10(2)
Gerald	24.35	6.34(4)	1.72(3)	1.04(3)	0.00(1)
Mascani	27.22	0.44(3)	1.59(2)	0.89(2)	1.05(4)
Tardis	27.34	0.37(1)	1.12(1)	0.74(1)	0.76(3)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*

Table 3.16.b *Mean groat area (mm^2), cultivar superiority, static stability and ranks values of the four winter varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Area Groat (mm^2)	*Mean*	*Cultivar Superiority*	*Static Stability*	*Sensitivity*	*Mean square Deviation*
Varieties					
Balado	15.15	1.01(3)	2.00(4)	1.82(4)	0.98(4)
Gerald	13.59	3.64(4)	0.52(2)	0.85(3)	0.00(1)
Mascani	16.02	0.16(1)	0.30(1)	0.389(1)	0.44(2)
Tardis	15.01	0.93(2)	0.56(3)	0.78(2)	0.54(3)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*

Mean grain length also differed significantly between environments (p-value<0.001, two way ANOVA), which averaged over all varieties ranged from 10.7 mm to 12.8 mm across the 11 environments (table 3.14). Gerald (10.57 mm) not only had shorter grains than the other 3 varieties but also was the most stable across environments (table 3.17.a, figure 3.32). Gerald also had the shortest groats (table 3.15 and 3.17.b). Tardis not only had the longest grains (13.44 mm) but was the most sensitive to the environment (table 3.17.a). Mascani and Balado however had the longest groats across environments (table 3.17.b.

Table 3.17.a. *Mean grain length (mm), cultivar superiority, static stability and mean square deviation values of the four winter varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Length (mm)	*Mean*	*Cultivar Superiority*	*Static Stability*	*Sensitivity*	*Mean Square Deviation*
Varieties					
Balado	11.94	0.04(2)	0.84(3)	1.04(3)	0.87(4)
Gerald	10.68	1.07(4)	0.54(1)	0.84(1)	0.00(1)
Mascani	11.52	0.21(3)	0.97(4)	1.12(4)	0.33(2)
Tardis	12.03	0.01(1)	0.75(2)	0.10(2)	0.54(3)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*

Table 3.17.b *Mean groat length (mm), cultivar superiority, static stability and mean square deviation values of the four winter varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Length Groat (mm)	*Mean*	*Cultivar Superiority*	*Static Stability*	*Sensitivity*	*Mean Square Deviation*
Varieties					
Balado	7.14	0.02(2)	0.14(4)	1.66(4)	0.69(3)
Gerald	6.43	0.37(4)	0.04(2)	0.85(3)	0.00(1)
Mascani	7.18	0.02(1)	0.05(3)	0.78(2)	0.94(4)
Tardis	7.06	0.04(3)	0.03(1)	0.66(1)	0.51(2)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*

Mean grain and groat width differed significantly (p-value<0.001, two way ANOVA) between environments which averaged over all varieties, ranged from 2.94 mm at Throws farm and 3.43 mm at Lydbury across environments (table 3.14). There was also a significant (p-value<0.001) difference between varieties with Mascani (3.23 mm) having wider grain than Tardis and Balado and all greater than Gerald (3.09 mm) (table 3.15). There was also a significant difference between varieties in their sensitivity to environment (table 3.18.a and 3.18.b). Balado more sensitive to the environment than the other three varieties, whilst Mascani and Tardis were the most stable Gerald had the lowest values in all size traits with means of 24.8 mm^2 area, width 3.00 mm and length, 10.95 mm.

Table 3.18.a. *Mean grain width (mm), cultivar superiority, static stability and mean square deviation values of the four winter varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Width (mm)	*Mean*	*Cultivar Superiority*	*Static Stability*	*Sensitivity*	*Mean Square Deviation*
Varieties					
Balado	*3.12*	*0.007(3)*	*0.024(4)*	*1.435(4)*	*1.382(4)*
Gerald	*3.06*	*0.013(4)*	*0.010(2)*	*0.931(3)*	*0.545(2)*
Mascani	*3.18*	*0.001(1)*	*0.007(1)*	*0.699(1)*	*0.327(1)*
Tardis	*3.12*	*0.005(2)*	*0.011(3)*	*0.902(2)*	*0.727(3)*
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*

Table 3.18.b *Mean groat width (mm), cultivar superiority, static stability and mean square deviation values of the four winter varieties. *Numbers in brackets refers to the position on the ranking of best cultivar.*

Width Groat (mm)	*Mean*	*Cultivar Superiority*	*Static Stability*	*Sensitivity*	*Mean Square Deviation*
Varieties					
Balado	2.514	0.013(4)	0.021(4)	1.403(4)	1.691(3)
Gerald	2.533	0.007(3)	0.007(1)	0.834(2)	0.564(2)
Mascani	2.624	0.001(1)	0.008(2)	0.733(1)	0.418(1)
Tardis	2.536	0.006(2)	0.009(3)	0.990(3)	0.564(2)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*

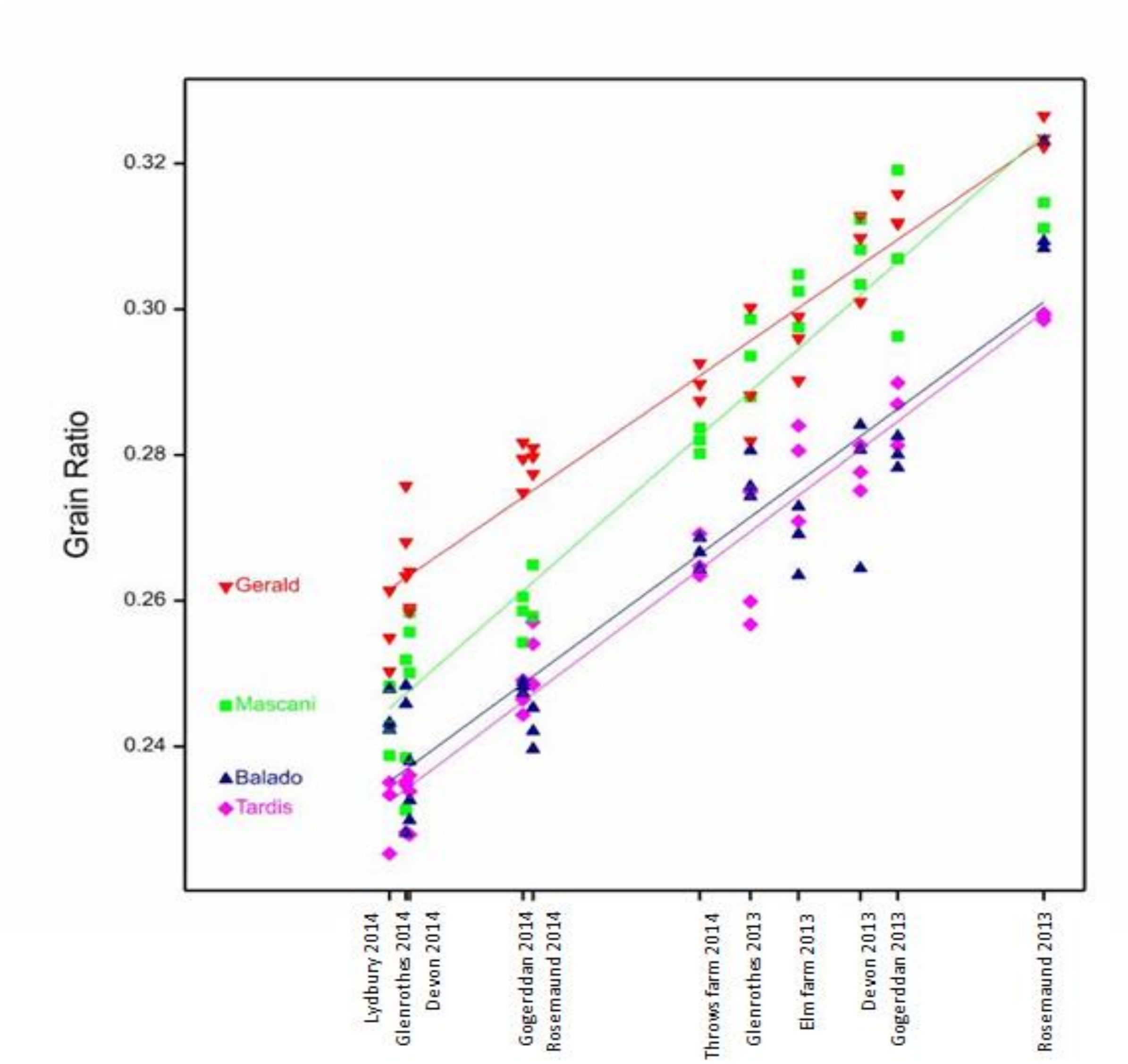


Figure 3.32. *Joint regression graph for grain ratio values of the four winter oat varieties and the eleven sites across UK, for 2012- 2013 and 2013-2014 harvest seasons*.

Joint regression analysis showed statistically significant sensitivity values (p-value<0.001) (figure 3.32), indicating that there is variation in genotype performance with changing environments for grain ratio. Cultivar coefficient, static stability, sensitivity values and mean square deviation values (table 3.19) showed Gerald with the highest mean and most stable independently of the environment whilst Mascani had the highest interaction with the environment.

Table 3.19. *Mean grain ratio, cultivar superiority, and static stability and mean square deviation values of the four winter varieties. *Numbers in brackets refers to the position on the ranking of best cultivar*

Grain Ratio	*Mean*	*Cultivar Superiority*	*Static Stability*	*Sensitivity*	*Mean Square Deviation*
Varieties					
Balado	0.263	0.0003(3)	0.0005(3)	1.061(3)	0.00007(2)
Gerald	0.288	0.0000(1)	0.0004(1)	0.805(1)	0.00003(1)
Mascani	0.278	0.0001(2)	0.0008(4)	1.312(4)	0.00005(4)
Tardis	0.261	0.0004(4)	0.0005(2)	0.816(2)	0.00002(3)
Significance	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*	*p-value<0.001*

In addition, frequency distribution analysis of the individual grain and groat data was conducted. These were analysed to determine their bi-modality and to establish the mean, standard deviation and the numerical balance between any subpopulations observed (Symons & Fulcher, 1988b). A bimodal frequency distribution was found regarding grain and groat area and length representing the primary and secondary grain found in each oat spikelet (figure 3.33). A two-normal distribution was fitted to these curves enabling the calculation of the mean and standard deviation of the primary and secondary grain subpopulations along with the proportions of grain in each (table 3.20), for all environments.

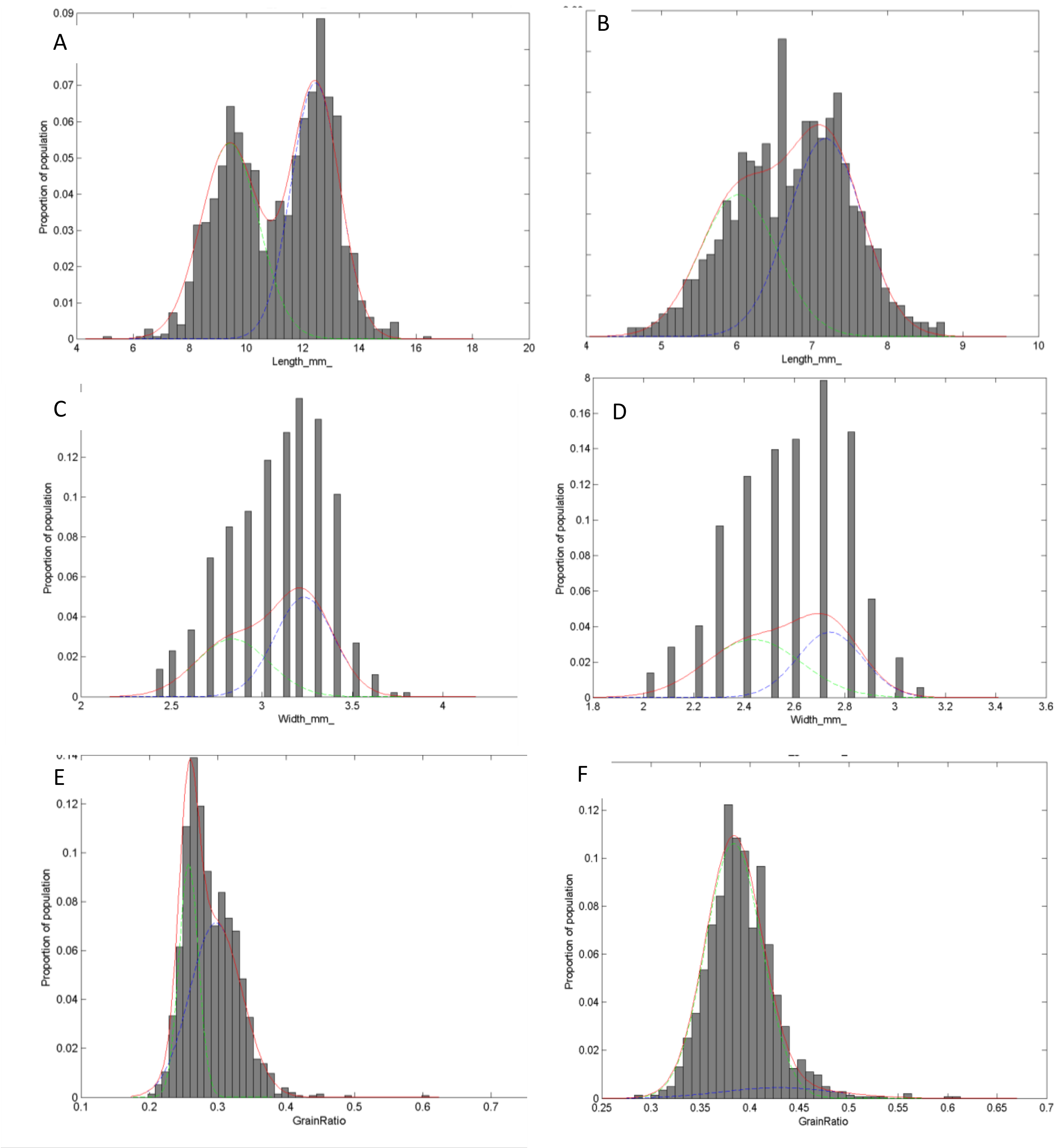


Figure 3.33. *Frequency of individual grain and groat length, width and ratio for grain and groats of Gerald grown at Glenrothes in 2013. A. grain length; B groat length; c, grain width; D groat width, E grain ratio; F, groat ratio. The fitted bimodal distribution is indicated in red with the primary grain distribution indicated in blue and secondary grain distribution indicated in green.*

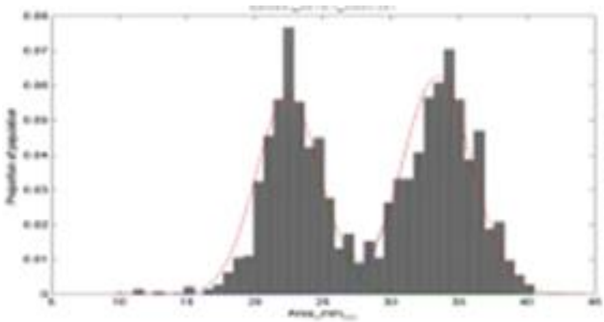
Tables 3.20 *Primary and secondary grain and groat length (mm) bimodality proportions by variety at each location and harvest season*

		Balado				Gerald				Mascani				Tardis			
		Grain		Groat		Grain		Groat		Grain		Groat		Grain		Groat	
		1^0	2^0	1^0	2^0	1^0	2^0	1^0	2^0	1^0	2^0	1^0	2^0	1^0	2^0	1^0	2^0
Gogerddan	2013	0.50	0.51	0.25	0.75	0.55	0.45	0.43	0.57	0.63	0.37	0.50	0.50	0.42	0.58	0.14	0.86
Devon	2013	0.54	0.46	0.36	0.64	0.55	0.45	0.32	0.68	0.49	0.51	0.41	0.59	0.49	0.51	0.12	0.88
Glenrothes	2013	0.48	0.52	0.29	0.71	0.58	0.42	0.53	0.47	0.62	0.39	0.40	0.60	0.38	0.62	0.16	0.84
Rosemaund	2013	0.38	0.62	0.18	0.82	0.64	0.36	0.38	0.62	0.45	0.55	0.70	0.30	0.29	0.71	0.95	0.05
Elm Farm	2013	0.51	0.49	0.34	0.66	0.54	0.46	0.41	0.59	0.51	0.49	0.49	0.51	0.45	0.55	0.23	0.77
Gogerddan	2014	0.66	0.34	0.30	0.70	0.53	0.47	0.47	0.53	0.54	0.46	0.51	0.49	0.38	0.62	0.15	0.85
Devon	2014	0.36	0.64	0.20	0.80	0.51	0.49	0.42	0.58	0.48	0.52	0.34	0.66	0.44	0.56	0.84	0.16
Glenrothes	2014	0.62	0.38	0.47	0.53	0.57	0.43	0.53	0.47	0.59	0.41	0.57	0.43	0.55	0.45	0.21	0.79
Rosemaund	2014	0.48	0.52	0.33	0.67	0.57	0.43	0.51	0.49	0.45	0.56	0.35	0.65	0.43	0.57	0.17	0.83
Lydbury	2014	0.46	0.54	0.39	.61	0.53	0.47	0.39	0.61	0.52	0.49	0.51	0.49	0.49	0.51	0.39	0.61
Throws Farm	2014	0.48	0.52	0.32	0.69	0.52	0.48	0.37	0.63	0.54	0.46	0.48	0.52	0.38	0.63	0.09	0.91
Mean		0.50	0.50	0.31	0.69	0.55	0.45	0.43	0.57	0.53	0.47	0.48	0.52	0.43	0.57	0.31	0.69

A less clear bimodal distribution was obtained for grain and groat width and grain and groat ratio (figure 3.33). An overlap was found between primary and secondary in terms of width and area for both grain and groat. Circularity and compactness did not show bimodal distribution for any variety or site.

An example of an interesting comparison of the bimodal distribution of grain and groat areas is shown in figures 3.34 and 3.35 and table 3.20, from Devon 2013/2014 harvest season. Whereas for the grain area distribution of all 4 varieties a clear bimodal distribution was obtained, when the groats are examined it is apparent that for all varieties except for Mascani there is not such a clear distinction between the primary and secondary populations. A far higher proportion of groats were found in the secondary distribution than would be predicted by looking at the distribution of grain size. For Balado across sites, 50% of the grain was in each of the primary and secondary distributions whereas for the groats, only 31 % were in the primary distribution was 53% and of groats it was 48%. This increase in the proportion of grain in the secondary distribution once the husk is removed was highest in Tardis, although for 2 sites this resulted in difficulties in fitting two normal distribution curves (table 3.20).

A



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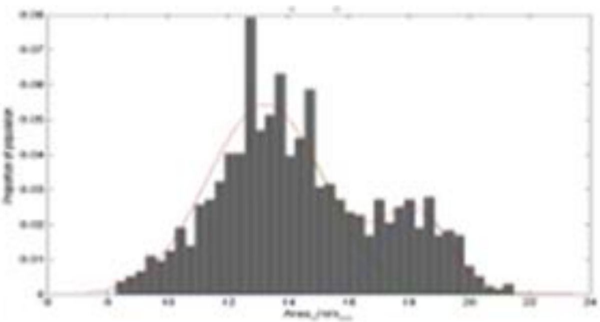
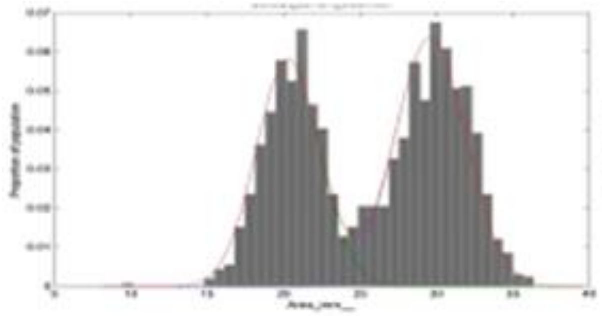


B



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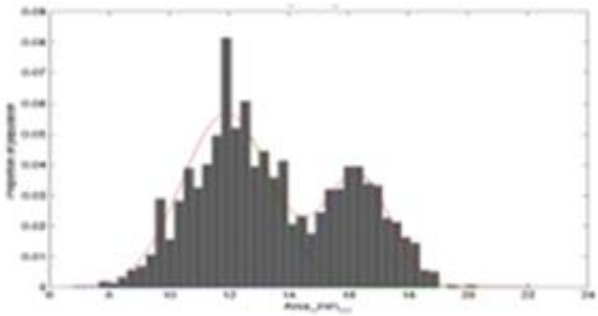

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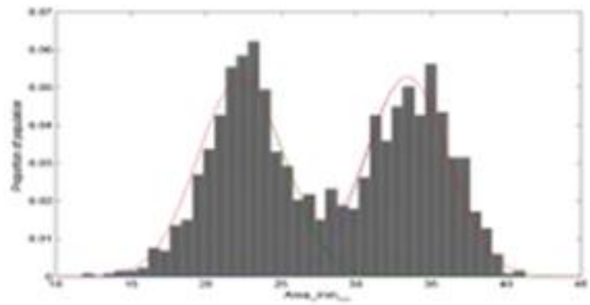
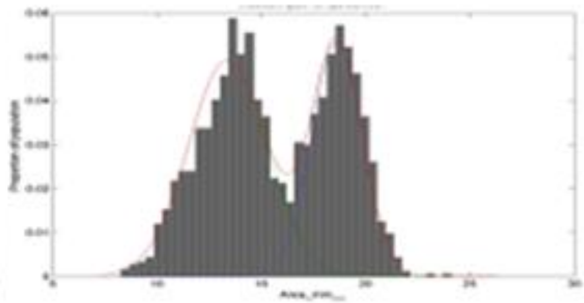
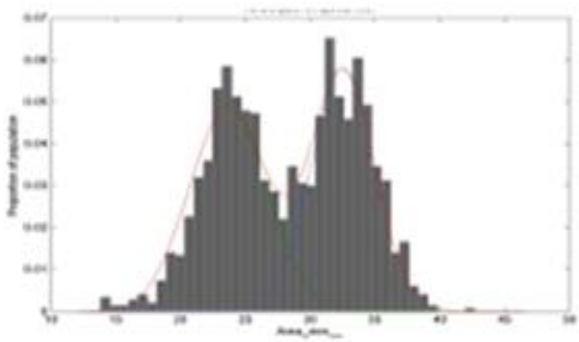


G



D



H

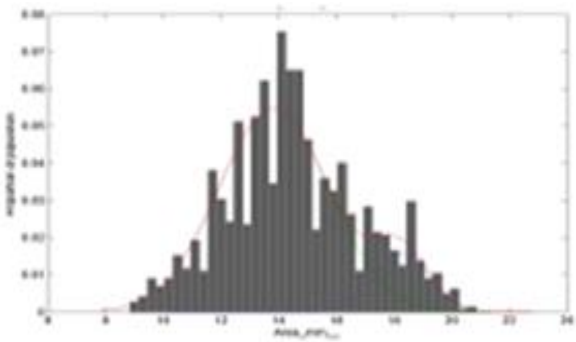


Figure 3.34 Grain (A - D) and groat (E - F) area (mm^2) bimodality graphs from Devon trial 2013 harvest season of Balado (A, E), Gerald (B, F), Mascani (C, G) and Tardis (D, H).

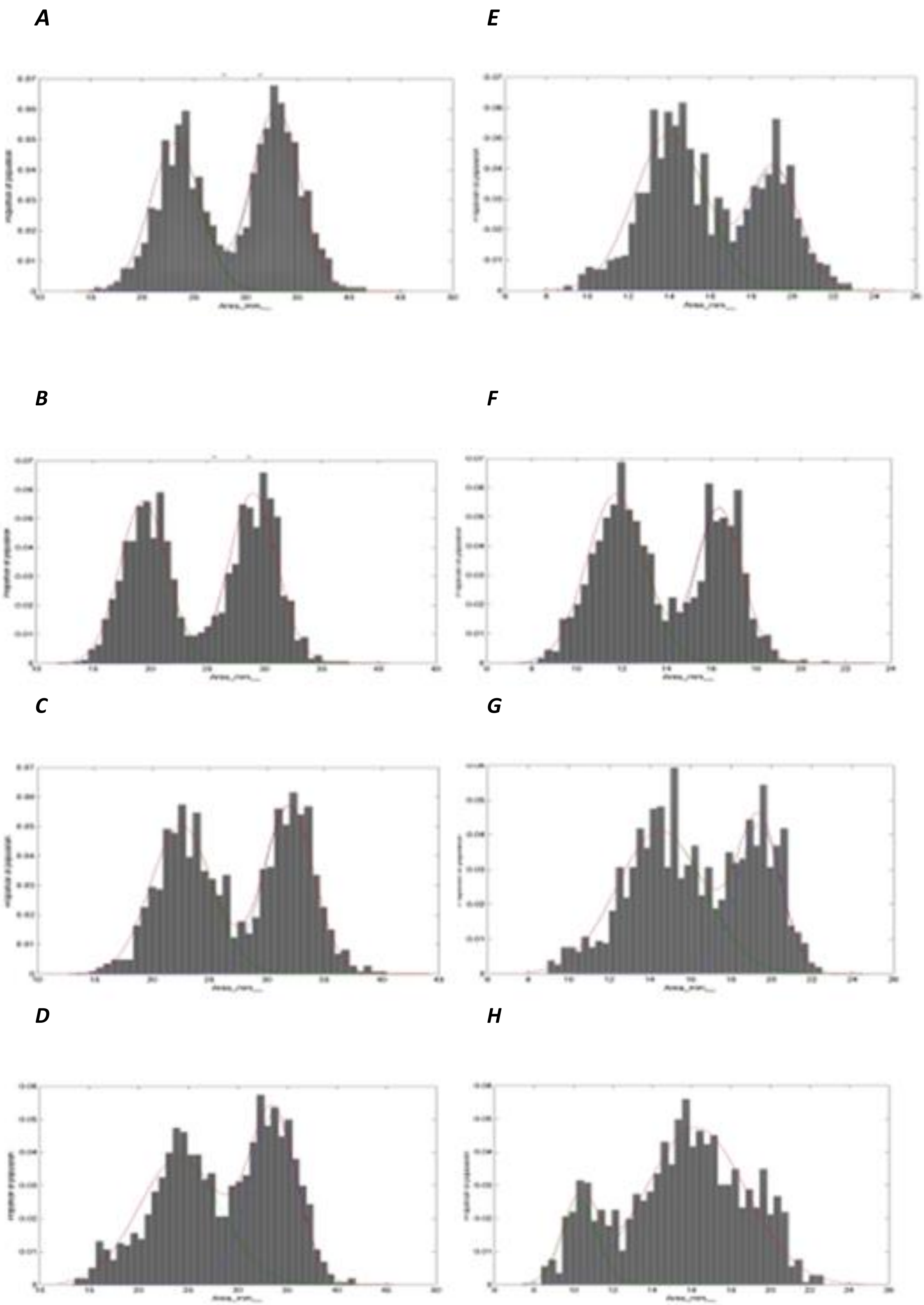


Figure 3.35 Grain (A - D) and groat (E - F) area (mm^2) bimodality graphs from Devon trial 2014 harvest season of Balado (A, E), Gerald (B, F), Mascani (C, G) and Tardis (D, H).

3.3.11 Correlations

The physical quality traits, i.e. kernel content (%), thousand grain weight, specific weight (t/hl) and hullability (%), were significantly correlated (p-value<0.05) with several grain and groat size parameters (table 3.21). Only those physical and chemical quality parameters with a correlation coefficient higher than an absolute value of 0.55 were considered.

Thousand grain weight was positively correlated (p-value<0.05), table 3.21 and figure 3.36, with grain area (mm^2), width (mm) and length (mm), with correlation coefficients in all cases above 0.70. The correlation was higher between grain width and thousand grain weight than with grain length and thousand grain weight. Similar correlations were found between groat dimensions and thousand grain weight.

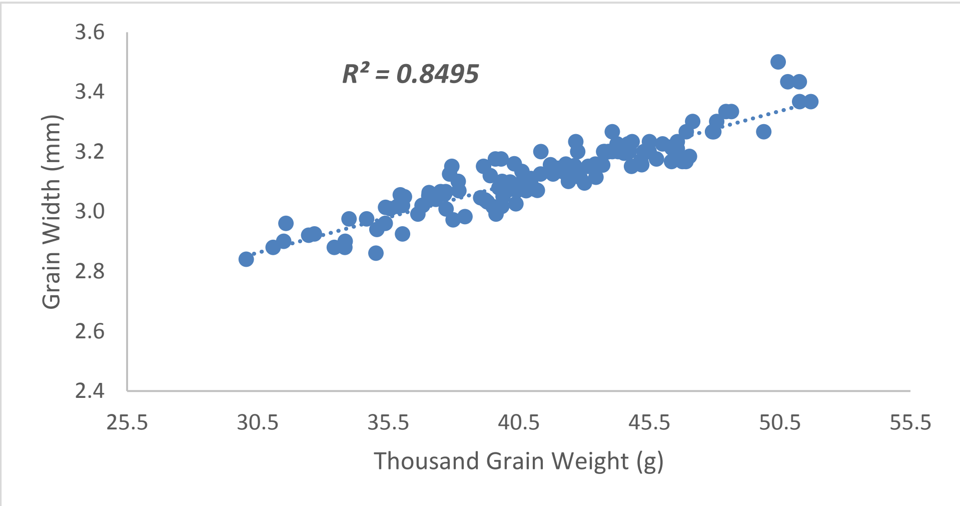


Figure 3.36. *Correlation plot between grain width (mm) and thousand grain weight (g) of the four winter oat varieties and the eleven sites across UK for 2012/2013 and 2013/2014 harvest seasons.*

This positive correlation was also found when varieties were examined individually (see appendix). Balado and Gerald showed consistently positive correlations between thousand grain weight and groat area, width and length, with correlation coefficients above 0.70. However, Mascani only presented this positive correlation in case of groat width. Tardis, on the other hand, showed significant positive correlations between TGW and with groat area, grain and groat width, grain and groat ratio and grain density.

Kernel content was also positively correlated (p-value<0.001) with grain width, groat area and width (figure 3.37) and thousand grain weight, although with a lower correlation coefficient (table 3.21).

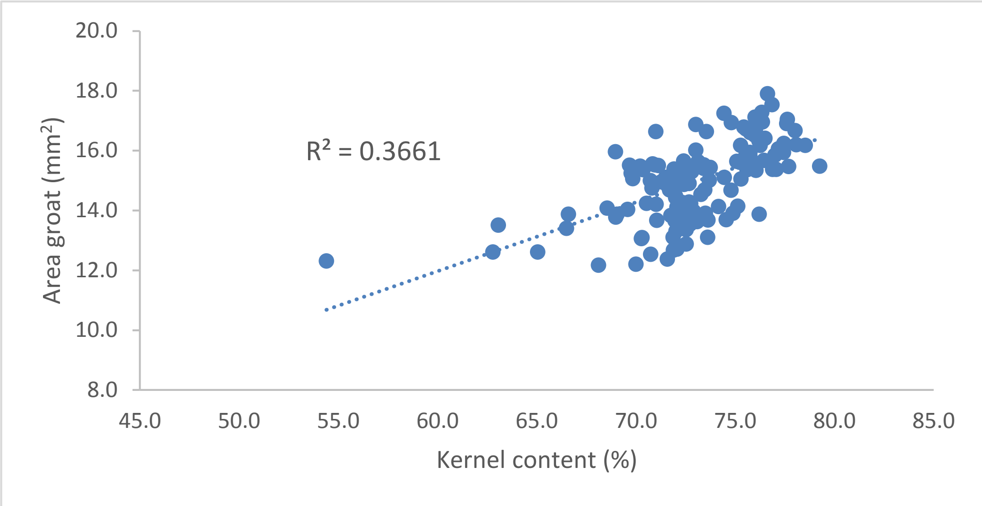


Figure 3.37. *Correlation plot between kernel content (%) and groat area (mm^2) of the four winter oat varieties values.*

When varieties were examined individually, Balado had similar results as mentioned above for overall kernel content values (figure 3.37). Gerald showed a positive correlation between kernel content and thousand grain weight and groat area and a negative correlation with grain number per metre square. Mascani´s kernel content, on the other hand, had a significant negative correlation with yield and grain number per meter square. Tardis however did not display significant correlations, either positive or negative, between grain size parameters and kernel content.

Hullability displayed a positive significant correlation (p-value<0.05), with grain density (table 3.21, figure 3.38). When varieties were examined individually, the hullability of Balado was positively correlated with groat length and negatively with yield and grain number per metre square. No statistically significant correlations were found with the hullability of Gerald, Mascani and Tardis when examined on a variety basis with any trait measured.

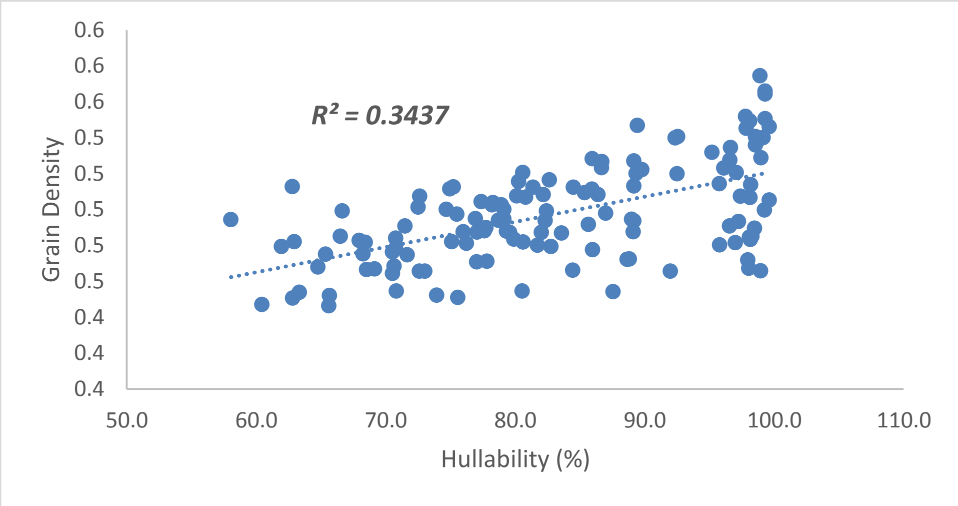


Figure 3.38. *Correlation plot between kernel content (%) and specific weight (kg/hl) of the four winter oat varieties values.*

Specific weight was significantly (p-value<0.05) correlated with grain density, grain ratio and groat width, groat ratio and circularity of the groat. However, the strongest correlation (table 3.21 and figure 3.39) was found between specific weight and groat width.

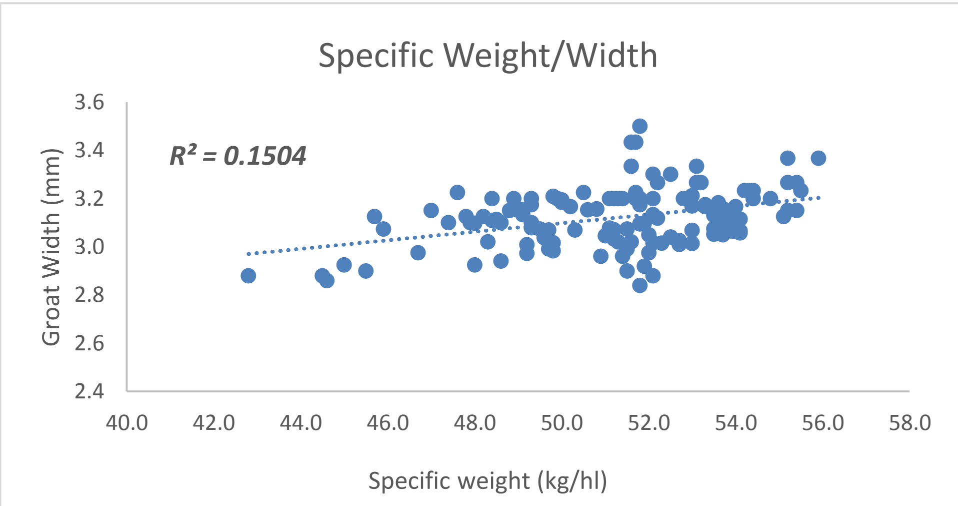


Figure 3.39. *Correlation plot between specific weight (kg/hl) and* groat width *of all varieties´ values.*

Tables 3.21. *Pearson´s linear correlation coefficients (p-value<0.05) between quality traits and grain and groat size of the four winter oat varieties. Green numbers show strong negative correlations whilst red numbers show strong positive correlations (p-value<0.001) for a two-tailed Pearson correlation test). Given the size of the table, it is split in three sections.*

	Oil	***Protein***	***B-Glucan***	***Kernel content***	***Hullability***	***Specific weight***	***Yield***	***TGW***
Oil	1.00							
Protein		1.00						
B-Glucan			1.00					
Kernel content	-0.71			1.00				
Hullability	-0.66			0.68	1.00			
Specific weight			-0.52	0.58		1.00		
yield (t/ha)							1.00	
TGW				0.59				1.00
Grain Density				0.47	0.59	0.48		0.57
grain n°/m^2	0.56			-0.59			0.85	-0.59
Area								0.66
Width				0.53				0.92
Length								
Circularity Grain								
Compactness								
Grain Ratio						0.46		
Area Groat	-0.53			0.61				0.89
Width Groat				0.60		0.58		0.82
Length Groat			0.50					0.68
Groat Ratio		-0.55	-0.61			0.57		
Circularity Groat		-0.56	-0.59			0.57		

Tables 3.21.b *Pearson´s linear correlation coefficients (p-value<0.05) between quality traits and grain and groat size of the four winter oat varieties. Green numbers show strong negative correlations whilst red numbers show strong positive correlations (p-value<0.001) for a two-tailed Pearson correlation test). Given the size of the table, it is split in three sections.*

	Grain Density	***Grain n^0/m^2***	***Area***	***Width***	***Length***	***Circularity Grain***	***Compactness***	***Grain Ratio***
Grain Density	1.00							
Grain n^0/m^2		1.00						
Area		-0.52	1.00					
Width	0.58	-0.49	0.48	1.00				
Length	-0.63		0.83		1.00			
Circularity Grain	0.79		-0.61		-0.94	1.00		
Compactness	-0.78		0.62		0.95	-1.00	1.00	
Grain Ratio	0.81		-0.56		-0.91	0.98	-0.97	1.00
Area Groat		-0.64	0.75	0.75				
Width Groat	0.68			0.91				0.54
Length Groat		-0.62	0.83	0.45	0.62			
Groat Ratio			-0.59		-0.72	0.70	-0.69	0.75
Circularity Groat	0.47		-0.59		-0.72	0.70	-0.69	0.74

	Area Groat	***Width Groat***	***Length Groat***	***Groat Ratio***	***Circularity Groat***
Area Groat	1.00				
Width Groat	0.69	1.00			
Length Groat	0.90		1.00		
Groat Ratio			-0.73	1.00	
Circularity Groat			-0.72	0.98	1.00

When examined by varieties, Gerald showed significant positive correlations between specific weight and thousand grain weight and grain and groat width, with values above 0.67 in all cases (see appendix). Thousand grain weight was positively correlated with specific weight values for Balado. Neither Mascani nor Tardis presented statistically significant correlations between specific weight and any other quality parameter or grain groat size and shape trait.

Chemical composition traits, i.e. oil, protein and b-glucan content, had diverse results (table 3.21). Oil content was significantly and negatively correlated with kernel content (%) and hullability (%) (p-value<0.001). By varieties Balado, Mascani and Tardis´s oil content showed a negative correlation with β-glucan content (%) (p-value<0.001), whilst Gerald along with Mascani and Tardis´ oil content was negatively correlated with grain length (p-value<0.001). The four varieties showed a negative correlation between oil content and grain area (p-value<0.001), figure 3.40.

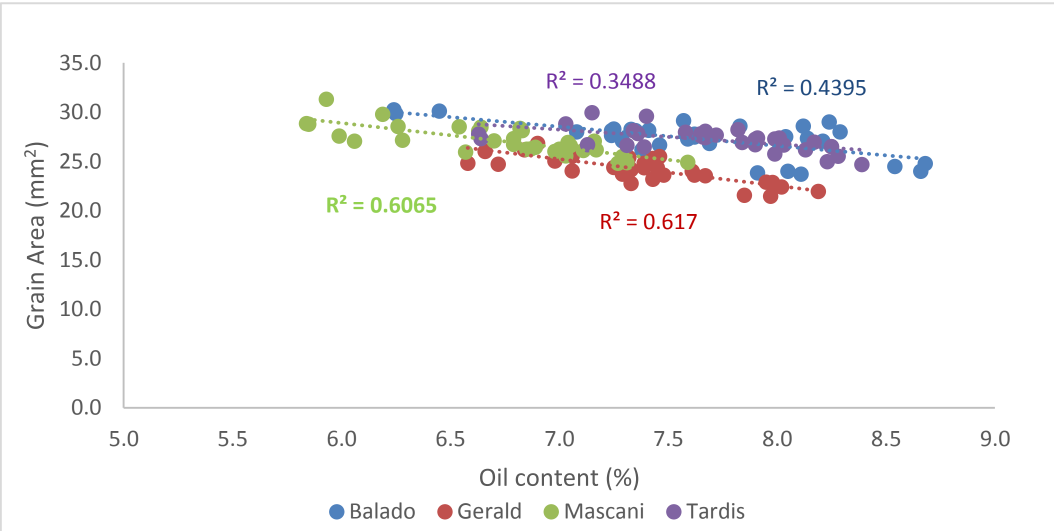


Figure 3.40. *Correlation plot between oil content (%) and thousand grain weight (g) of all varieties´ values.*

Protein content had significant negative correlations (p-value>0.05) (table 3.21), with groat ratio and circularity of the groat. When analysed by varieties, all of them showed a significant (p-value<0.05) negative correlation with groat ratio, figure 3.41. Mascani also presented a significant (p-value<0.05) positive correlation with grain width.

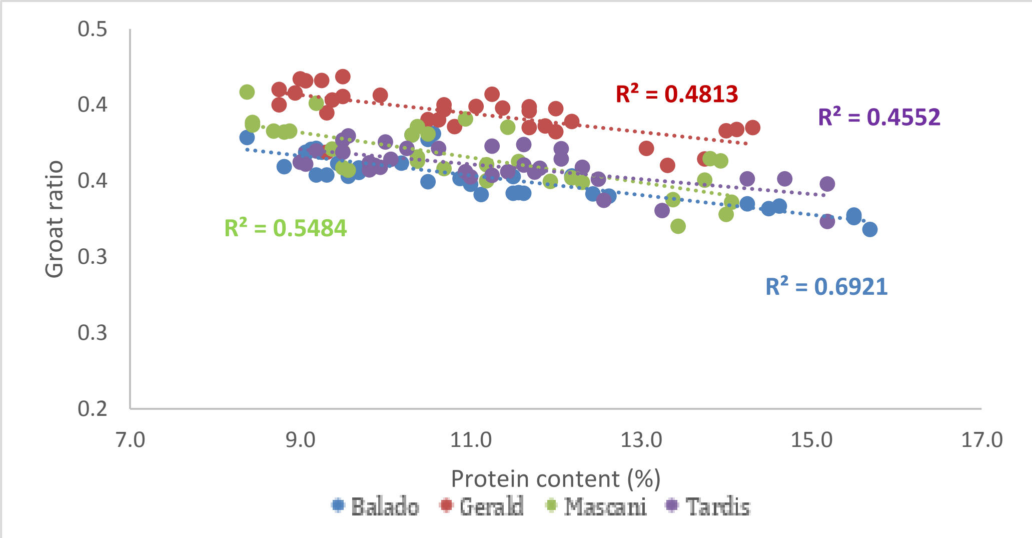


Figure 3.41. *Correlation plot between protein content (%) and groat ratio of all varieties´ values.*

B-glucan content (%), presented a significant (p-value<0.05) negative correlation (table 3.21) with groat ratio (figure 3.42), likewise to the negative correlation found for protein content by varieties. Gerald did not show any significant (p-value<0.05) correlation, either positive or negative, with any of the quality traits.

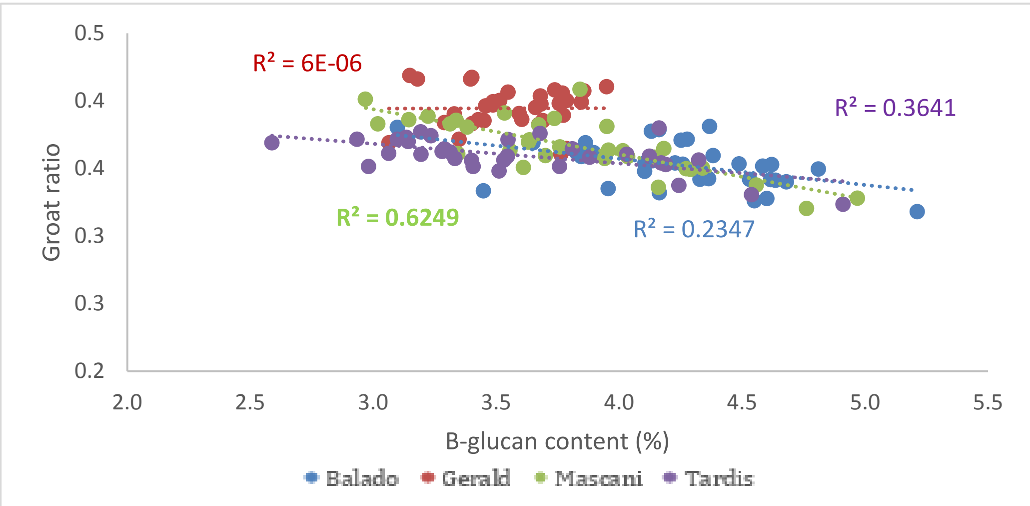


Figure 3.42. *Correlation plot between β-glucan content (%) and groat ratio of all varieties´ values.*

On the other hand, b-glucan content was significantly (p-value<0.001) and positively correlated with hullability (%), grain length and groat area (mm^2) for Balado. Mascani also showed b-glucan content negatively (p-value<0.001) correlated with grain width (mm) and grain and groat ratio (green line and R square, figure 3.42), while positive with grain length (mm).

3.4 Discussion

Plant breeders aim to develop improved crop varieties that are adapted to produce high yields of quality grain over a wide range of environments (Doehlert, 2001) with the adaptability of a variety usually tested by the degree of interaction with different environments under which it is planted (Ashraf, Qureshi, Ghafoor, & Khan; (2001); Asif, Mustafa, Asim & Mujahid, 2003; Sial, Arain & Ahmad, 2000).

Analysis of the genotype by environment interaction on grain yield and quality is therefore essential in variety evaluation (Becker & Leon, 1988; Subira, Garcia del Moral, & Royo, 2015) and to understand the adaptability and stability of varieties (Hongyu, Garcia-Pena, Borges de Araujo & Tadeu dos Santos Dias, 2014) for different environments. The GxE effects on selected oat grain quality traits (Doehlert, 2001) and on β-glucan content in commercially available varieties (Andersson & Börjesdotter, 2011) and within related wild species (Redaelli, Del Frate, Bellato, Terracciano, Ciccoritti, Germeier, De Stefanis, & Sgrulletta, 2013) have been studied, but limited information exists for other grain components or on milling quality traits.

Historical data analysed graphically showed that a high degree of variability is apparent between years and between trial sites as well as between varieties. All the historical data were obtained from recommended list trials which all received a similar agronomy suggesting that this variability might be explained by the different environments where the different varieties were grown and by the climate variability between sites.

In this study none of the varieties displayed a superior performance in all quality traits neither did one site show superior performances over all values for all varieties. However, there were statistically significant differences between varieties and across the eleven environments over the two harvest seasons, and significant interactions between genotype by environment, when analysing yield, specific weight, kernel content, hullability, thousand grain weight, grain and groat composition and grain and groat size. Yield was significantly different across sites but not between varieties showing more environmental influence on yield than genotype. Other studies have found strong correlations between kernel content and yield (Achleitner et al., 2008; Doehlert, 2001). However, the weak correlation found in this study is in accordance with those reported Forsberg & Reeves, 1995.

By sites and traits, Rosemaund yielded the highest hullability, kernel content and β-glucan content for both harvest seasons and the lowest oil content. However, it showed the lowest specific weight in 2014 harvest season. Rosemaund 2013 field trial was sown in February. A shorter period of plant development in comparison with the rest of the field trials and seasons, affecting therefore, grain and groat size and shape, might explain the differences found in specific weight, oil, β-glucan content and kernel content, between harvest seasons at Rosemaund. Devon had the lowest hullability values in 2013; however, its value in 2014 was above 80 %. Gogerddan 2013 and 2014 showed the lowest protein content but the highest oil content. Throws Farm 2014 reached the lowest kernel content and the smallest grain, whilst Lydbury 2014 yielded the highest specific weight and thousand grain weight. Elm Farm, Glenrothes 2013 and 2014, showed variable levels in terms of hullability, kernel content, specific weight, thousand grain weight, oil and protein content. While Elm Farm had low hullability, kernel and oil content, Glenrothes showed high hullability, kernel content, specific weight and oil content.

Differences in trait values found might allow us to think that these sites are more suitable to investigate differences in terms of genotype by environment interactions, considering at the same time, the differences in management conditions between both sites. However, given that only one season of data from Elm farm 2012/2013 trial under organic management conditions were available no conclusions can be drawn on the effect of management on yield and grain quality. Glenrothes showed variable quality trait values in both harvest seasons with low specific weight and kernel content in 2013, contrasting with specific weight and kernel content from 2014. This allows us to conclude a greater effect of year, i.e. climate/weather conditions and a different interaction with the environment between both seasons.

Table 3.22 *Summary table of genotype, environment and genotype by environment interactions effects on grain and groat quality parameters for the milling industry and end-users, from joint regression analysis.*

Quality parameter	*Genotype by Environment Interaction*	*Genotype*	*Environment*
Yield (t/ha)	<0.001	non-significant	<0.001
Kernel content (%)	<0.001	<0.001	<0.001
Specific Weight (kg/hl)	non-significant	<0.001	<0.001
Hullability (%)	<0.001	<0.001	<0.001
Thousand Grain Weight (g)	<0.001	<0.001	<0.001
Oil content	<0.001	<0.001	<0.001
Protein content	<0.001	<0.001	<0.001
β-Glucan content	<0.001	<0.001	<0.001
Grain area (mm^2)	<0.001	<0.001	<0.001
Grain length (mm)	<0.001	<0.001	<0.001
Grain width (mm)	<0.001	<0.001	<0.001

Specific weight is a controversial trait. Some studies have found it highly heritable and positively correlated with kernel content (Forsberg & Reeves, 1995; Doehlert, McMullen & Baumann, 1999; Doehlert, 2001). Although some other studies conducted in wheat have shown a poor relationship between specific weight and other quality traits (Wilkinson *et al.*, 2003; Owens *et al.*, 2007), in this research specific weight values, were statistically significant between environments and between varieties (table 5.1), Mascani being the most stable and with the highest values across sites. However, the grains with the highest specific weight were those that were smallest, i.e. Gerald, in accordance with previous research (Peterson & Wood, 1997). There was no significant difference for interaction of genotype with the environment for specific weight. Thus, the significant variation found in specific weight values, might be explained because of the differences between genotypes under study rather than because of the interaction of the genotype with the environment. There was also a significant positive correlation between specific weight and kernel content. Although this correlation was not strong (0.58 Pearson correlation coefficient (r), p-value<0.001) it might be possible to select for varieties or genotypes with the best values of both traits.

Varieties´ hullability (%) and kernel content (%) showed variable results. Mascani showed the highest values in kernel content, hullability and thousand grain weight, with superior cultivar values and static stability and the lowest sensitivity to the environment. These remarkable superiority and stability values suggest Mascani (Gogerddan, 2004; Griffiths *et al.*, 2008; White & Watson, 2010) as the most suitable variety to continue further investigations including more sites and harvest seasons to find out differences between Mascani and others varieties and therefore, allow to select by breeding for those quality characteristics. Tardis had the highest protein content (%), whilst Gerald, although it had highest specific weight (kg/hl), showed the lowest thousand grain weight (g) and β-glucan content (%). Overall by seasons and sites Balado showed the highest β-glucan content (%).

To evaluate the stability of a genotype across environments a number of different indices were calculated using the data obtained here. Regarding stability, the static concept refers to the ability of a genotype to perform consistently across different environmental conditions while the dynamic (or agronomic) concept of stability implies that a stable genotype shows a yield response in each environment that is always parallel to the mean response of the tested genotypes (Becker & Leon, 1988). Joint regression analysis (Finlay & Wilkinson, 1963) enables the calculation of both the sensitivity of a genotype to the environment and also the mean square deviation from that regression line. Non-parametric phenotypic parameters, i.e. cultivar superiority, ranks and static stability, provided a useful alternative, reducing outliers, being easy to use and interpret, and not needing distribution assumptions. However, these non-parametric phenotypic parameters still need to elaborate efficient tests of significance, the theoretical relationships between all of these parameters and the classic regression approach (Finlay & Wilkinson, 1963; Huehn, 1990). In general, the ranking of the superiority index matched that of the mean values obtained for each trait.

The static stability, sensitivity and mean square deviation values also provided similar rankings to each other. This was not always the case as shown by the values for protein content for Tardis which had the lowest stability and sensitivity values but the highest mean square deviation value indicating that although it had low sensitivity to the environment but it gave the least predictable response. For any given trait, a stable variety does not necessarily also have high mean performances although this was found for many traits in this study. For two traits (β-glucan content and grain length), the most stable variety was Gerald but it had the lowest mean values.

Kernel content, hullability and thousand grain weight displayed statistically significant interactions with the environment. Lydbury 2014 showed higher values for the three quality traits mentioned (table 3.12). However, removing the environmental effect (figure 3.20), the range of quality trait values displayed allow us to discard this site as the most effective to differentiate between genotypes (Mcdermott & Coe, 2012).

Chemical composition suggest Throws farm 2014 as the best site for better results in oil and protein content, although with not the overall mean highest β-glucan content. Rosemaund 2013 displayed the highest β-glucan content overall mean and good levels of protein content but low oil content. All of the three chemical traits showed significant interaction with all the eleven sites under study as previously reported (Brunner & Freed, 1994; Doehlert, 2001; Peterson, 1991). Tardis was superior in comparison with the rest three varieties in terms of oil and protein content, although Balado displayed the highest β-glucan content. All varieties were statistically sensitive to the environment and according to stability non-parametric values Gerald is the most stable and giving predictable responses in terms of oil and protein content. This, along with the interaction between genotype and environment, suggest a niche-matching variety according to the quality trait of interest.

Image analysis of grain and groat size and shape confirmed a bimodality distribution frequency (Doehlert *et al.*, 2005; Wychowaniec et al., 2013). Primary and secondary grain and groat showed for all traits and in all varieties bimodal distributions regarding size and shape although the most apparent bimodal distribution was found for grain and groat length. Area, width, circularity and roundness showed a higher overlap of the two sub-populations. Grain and groat area showed a stronger variation along length, meaning a stronger correlation than area with width, for all harvest seasons and varieties. This result was also observed for grain and groat ratio, although the effect was not so strong. The highest specific weight grains were the smallest and shortest varieties, i.e. Gerald (Peterson & Wood, 1997).

These results might be explained by panicle development in oats. In oats spikelets comprise usually two to three grains (Welch, 1995) with the primary one larger in comparison with the secondary and the tertiary grain (Browne et al., 2002). The secondary grain is higher in both kernel content and hullability (Browne et al., 2002). Because of this particular structure, the subpopulation under the curve in the bimodality graph and the proportions calculated do not include primary or secondary grain exclusively but also a certain number of grains that should belong to one category or another, making it difficult to establish the limits between them. On the other hand, some of the results in the bimodality proportions calculated showed odd values, leading to the conclusion that further development in the mathematical method to assess those parameters is needed. Although further research is needed, the study of bimodality characteristics of grain and groat subpopulations in oats could lead to a new quality parameter (Symons & Fulcher, 1988a).

The positive and negative correlations found between oil, β-glucan and protein content are in accordance with previous research reported. Interestingly there was a mirror effect between oil and protein content, confirmed by the negative correlation found between them. These results are not conclusive nor do they allow establishing a causal relationship between the two parameters. Some reports suggest a different relationship between traits, making it difficult to establish causal reasons between traits. Similarities in grain size between Balado and Gerald and between Mascani and Tardis, i.e. length, area and width, could explain the similarities in coefficient correlations. The negative and positive correlations found between grain size and shape when studying each of the varieties, showed the influence of area (mm^2), length (mm) and width (mm), over each quality parameter under study.

The variability found between sites and years suggest that locally adapted varieties would perform better, i.e. niche-matching, therefore, choosing varieties according to the historical performance of the site rather than the overall performance of the variety. This would include taking in consideration not only mean values obtained in previous experimental trials, but parameters shown in this thesis. Static stability, sensitivity values and mean square deviation, would allow choosing a variety with predictable and consistent response across environments. On the other hand, sites with good overall performance in quality traits under study should be considered, to discriminate between varieties performances and thus dissect the basis, physic, genetic and environmental, of those differences (Becker & Leon, 1988; Mcdermott & Coe, 2012).

Because of management conditions (fertilizer levels, pest control intensities, irrigation levels) and differences in and within the field (Roel, Firpo & Plant, 2007), the variability between years and genotypes might be a factor explaining differences found.

Future challenges include the determination of how much of the observed quality traits' variability was caused by natural variation in yields and how much by differences in management practices, by analysing more data collected along more harvest seasons and across major areas of crop production. Therefore, it will be necessary to determine the best cost-effective management practices are most appropriate for what conditions, both edaphic and climatic, in the region to get the most of each variety. Future experimental trials should be designed to include major areas of crop production and different genotypes over longer periods of time, i.e. more harvest seasons, allowing a better understanding of genetic and environment interactions and their consequences. This would allow the development a niche-matching list of varieties across the country to which the farmer could refer when searching for an oat variety with higher results when farming in a certain area of the country.

3.5 Conclusions

- Yield was significantly different across sites but not between varieties showing more environmental influence on yield than genotype. A weak correlation between yield and the rest of milling quality parameters was found in this study.
- None of the varieties under study displayed a superior performance in all quality traits neither did one site showed superior performances over all values for all varieties.
- There were statistically significant differences between varieties and across the eleven environments over the two harvest seasons and significant interactions genotype by environment, when analysing yield, specific weight, kernel content, hullability, thousand grain weight, grain and groat composition and grain and groat size.

- The variability found for a given variety across harvest seasons and sites, suggests a niche-matching strategy, when cultivating in a specific area and/or for a specific market requirements.
- The positive correlations between milling quality parameters found in this research might allow for the development of selection tools based on just one of those correlated traits to breed for new varieties with enhanced milling industry and end-user requirements.
- The results from this research could help to develop a predictive model of grain quality parameters. This predictive model would be based on developing proxy measures with a strong effect on grain quality parameters.

Chapter Four. Applied nitrogen. Effects on grain quality parameters

4.1 Introduction

Increasing the competitiveness of oats among other cereals, requires a nitrogen optimum rate of fertilization that on one hand time minimises environmental impact and at the same time maximizes milling industry and farmer's benefits. To get maximum crop production it is essential that all nutrients are present through the season (Forsberg & Reeves, 1995). Oats are described a as low input cereal (Kindred et al., 2008) , needing lower nitrogen fertilizer compared with other cereals. For example, in the U.K. currently the recommendations are 160 kg ha $^{-1}$ nitrogen for winter oats compared to 250 kg ha $^{-1}$ for winter wheat (HGCA, 2009). Oats are grown mostly in temperate climates where the use of N fertilizer is the major factor affecting crop production. Although recent years have seen an increased number of experiments regarding nitrogen fertilizer effects on oats yield, milling quality parameters have not been the focus of investigations to date, in comparison with other crop species due to smaller contribution of oats to the total cereal production in the UK (Chalmers et al., 1998).

Nitrogen as fertilizer, along with phosphorus, are key nutrients limiting crop production although there is growing evidence that other nutrients such as sulphur and some micronutrients constrain production in cereal yield in non-fertilized agriculture influencing soil fertility (Kihara, Nziguheba, Zingore, Coulibaly, Esilaba, Kabambe, Njoroge, Palm & Huising, 2016). Even though nitrogen is widely present in nature, most soils lack sufficient biologically available nitrogen, and therefore an appropriate application of fertilizers is needed (Geleto, Tanner, Mamo, & Gebeyehu,1995). Management of fertilization conditions depend on yield goal and previous soil conditions, i.e. moisture content, kind of soil, previous crop, time of application and risk of lodging (Forsberg & Reeves, 1995). It is important that in addition to optimising grain yield that the end-use quality of that grain is also maximised. Nitrogen added to the soil increases final shoot number and therefore grain number per unit area whose positive correlation (Lawes, 1977; Browne et al., 2004; Browne et al., 2006) increasing yield. However, non-controlled fertilization levels applied might result in higher tiller numbers with a higher rate of competition between tiller growth and therefore, a shorter grain filling period and consequently poorer grain quality (Diekmann & Fischbeck, 2005). Also, higher levels in total nitrogen applied have been found to diminish individual grain weight (Peltonen-Sainio & Peltonen, 1995; Chalmers et al., 1998; Kindred *et al.*, 2008). At the same time, shorter grain filling period results in lower kernel content and hullability, and an increased number of screenings (Browne et al., 2004; Browne et al., 2006).

Timing of application, efficiency in the uptake, use and mobilisation of nitrogen by crops, are among others, key elements in the process. Protein grain yield depends on the plants ability to mobilize N uptake to the grain since most of the N applied is taken up by the plant before anthesis, accounting for more than a 75% of the remobilization and translocation of N from vegetative to reproductive tissue (Cataldo, Maroon, Schrader & Youngs, 1975; Rattunde & Frey, 1986). Applied at early stem extension, N results in improved tiller survival but an increased risk of leaching to the environment (Maidl, Sticksel, Retzer, & Fischbeck, 1998; Kindred et al., 2008; Zhao, Ma & Ren, 2009). Later in the season, it results in delayed senescence and longer grain filling season and grain number per ear in durum and winter wheat (Geleto et al., 1995; Maidl et al., 1998).

In oats, the effects of different levels and application timing of N is relatively unknown in terms of effects on grain quality parameters such as, grain size and shape, and variety response to nitrogen application. The interaction between cultivar and N levels of fertilization has been shown to affect yield, highlighting the importance of research on the effects of different levels of nitrogen fertilization on other key quality parameters (Ma et al., 2012).

In this research, the first objective was to investigate the effect of different levels of nitrogen fertilization on milling quality parameters and on grain size and shape traits. Secondly, focuses on to investigate the effect of increasing levels of nitrogen fertilizer on four winter oat varieties milling quality parameters, being the hypothesis that there are differences in the response between them. Thirdly, look at the interaction of different levels nitrogen fertilization and varieties on milling quality parameters, understanding stability to changes and the genetic importance on milling quality parameters by variety.

4.2 Material and methods

In the present study, the variability usually found because of environmental factors was reduced by performing replicated experiment at each location, in each harvest season, and focusing on N nutrition of the crop.

The experimental design included three sites in two harvest years. In 2013/2014 trials were grown at Lydbury North (latitude 52.45, longitude 2.94) and at Fawley Farm (latitude 51.98, longitude -2.60). These trials were managed by IBERS and ADAS respectively and are referred to throughout as IBERS14 and ADAS15. In a complete randomized experimental trial, four winter oat varieties, Balado, Gerald, Mascani and Tardis (for a description of the varieties see chapter two), were sown in 24 by 2-metre plots in 2013/2014 (figure 4.1), and in 12 by 2 metre plots in 2014/2015.

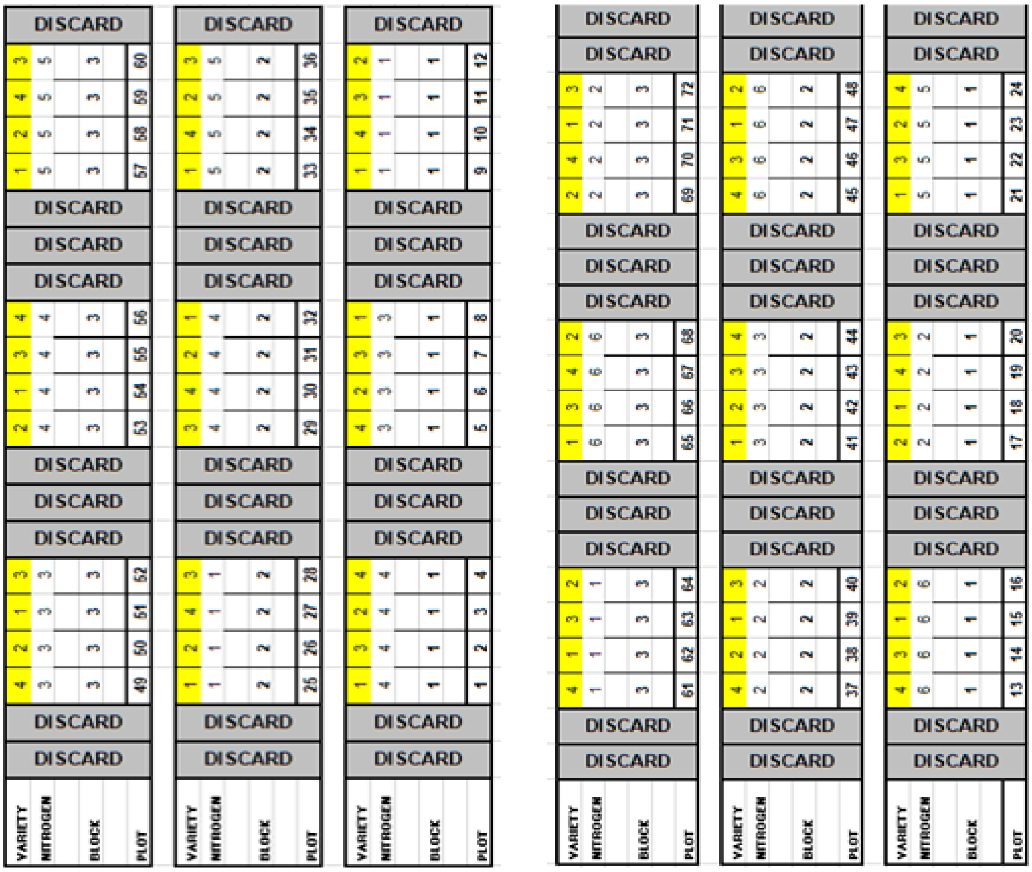


Figure 4.1. *Representation of trial field plan for ADAS 2014. Areas shaded with grey (DISCARD) represent the borders between nitrogen treatments blocks. Yellow blocks, numbered one to four represent each of the four varieties, Gerald (1), Mascani (2), Tardis (3) and Balado (4). White blocks numbered one to six, represents each of the six levels of nitrogen applied.*

Sowing dates were 30/09/2013 and 01/10/2014 at ADAS 2014 and 2015 respectively, whilst for IBERS 2014 it was 09/10/2013. Soils were sampled and classified in early spring for planting management conditions following standard procedures by each trial operator AHDB guidelines ('Section 4 Arable crops Nutrient Management Guide (RB209)', 2018). All trials were on soils described as "medium" which have a moderate ability to retain nitrogen and allow average rooting depth. The Soil Nitrogen Supply (SNS) and Soil Mineral Nitrogen (SMN) data in table 4.1 were used to calculate the amount of nitrogen applied (ADAS Rosemaund personal communication). SMN is defined as the proportion of soil nitrogen that is directly available to plants as nitrate or ammonium, together with an estimate of mineralisable nitrogen and crop content. The standard procedure for analysis of available soil nitrogen is well documented, and consists of extraction with KCl, filtration of the extract, analysis by colorimetry, and conversion of nitrate and ammonium ppm to kg/ha based on bulk density of the soil (Knight, 2006). The management and application conditions and dates are described in table 4.2 a, b.

Table 4.1. *Previous cropping and soil N assessment for the three N response trials (data supplied by ADAS and IBERS personal communication). *According to RB209 guidelines by DEFRA*

	Previous crop	***Soil class***	***SMN (kg N/ha)***	***Crop N***	***SNS***	***SMN Index***
IBERS ´14	Spring barley	Medium	56.0	15	111	3
ADAS ´14	Winter wheat	Medium	22.4	15	37.4	3
ADAS ´15	Winter wheat	Medium	24.0	15	39	3

Table 4.2.a *Nitrogen fertilizer applied (kg/ha), dates and doses of application in ADAS14 and ADAS15. The early stem extension 2nd split was applied 2 weeks after the 1st split.*

N LEVEL	*Early March application (kg N/ha)*		*Early stem extension 1st split (kg N/ha)*		*Early stem extension-2nd split (kg N/ha)*		*Total Nitrogen applied (kg N/ha)*	
	ADAS14	ADAS15	ADAS14	ADAS15	ADAS14	ADAS15	ADAS14	ADAS15
0	0	0	0	0	0	0	0	0
1	0	0	50	30	0	30	50	60
2	50	40	50	40	0	40	100	120
3	50	40	50	70	50	70	150	180
4	50	40	75	95	75	95	200	240
5	50	40	100	120	100	120	250	280

Table 4.2.b Nitrogen fertilizer applied (kg/ha), dates and doses of application at IBERS 2014/2015 harvest season

N LEVEL	*4th April application (kg N/ha)*	*17th April split (kg N/ha)*	*2nd May split (kg N/ha)*	*Total Nitrogen applied (kg N/ha)*
0	0	0	0	**0**
1	0	50	0	**50**
2	50	50	0	**100**
3	50	50	50	**150**
4	50	75	75	**200**

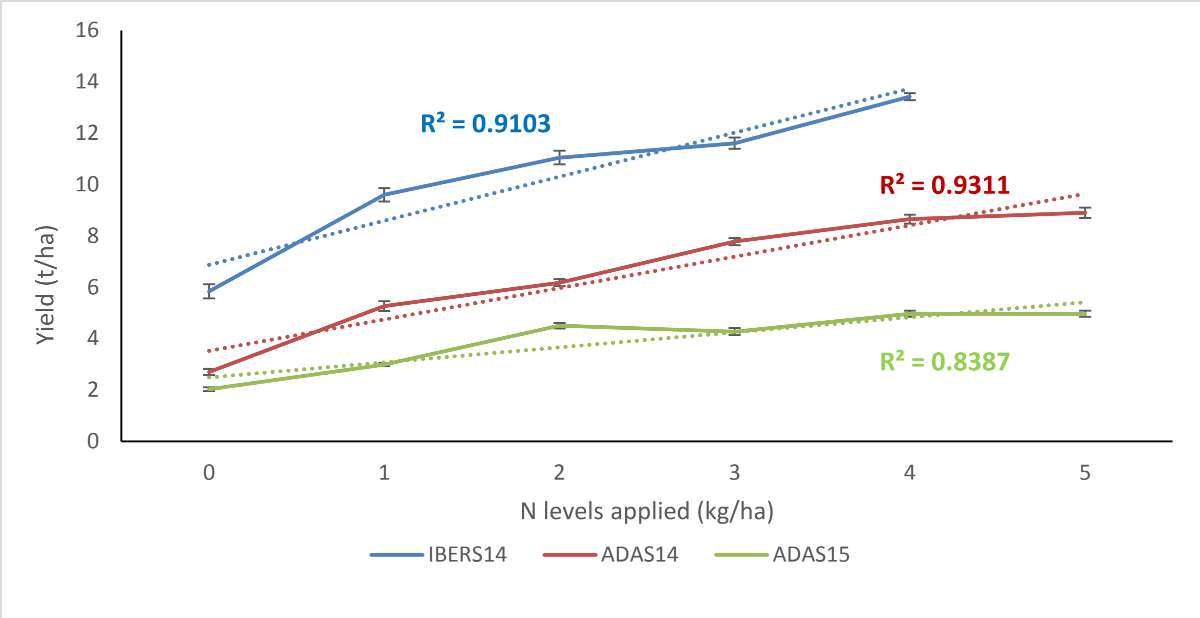
The treatments consisted of six levels of nitrogen applied at the ADAS sites, from zero to a total of 250 kg per hectare in 2014 and from zero to a total of 280 kg per hectare in 2015 (table 4.2a). The higher level of N applied at ADAS 2015 is justified because, when analysed, the preliminary yield results at ADAS 2014 at the highest level of N applied, i.e. 250 kg/ha, i.e. yield did not show a plateau. Thus, it was decided for ADAS 2015 experimental treatment to increase the levels of N applied. Five levels of nitrogen were applied from 0 to 200 kg per hectare at IBERS in 2014. This difference in doses of N applied, between IBERS 2014 and ADAS 2014 and 2015, were due to the higher presence of N in the soil at the start of the growing season (table 4.1). The fertiliser type chosen was in solid ammonium nitrate granules (about 34% N) (HGCA, 2009). The doses were split between three different development stages (table 4.2.a, b), to better timing and tailored to match crop demands, i.e. physiological demands at early uptake, stem extension, flowering time and grain filling.

Trials were harvested on 4/08/2014 for ADAS 2014 and 08/08/2015 for ADAS 2015 and 07/08/2014 for IBERS2014, respectively, and grain retained for analysis as described in chapter 2. Means and standard errors were calculated for graphical analysis. Pearson correlation tests were conducted between nitrogen levels and mean trait values. Joint Regression analysis for all data collected was developed with nitrogen levels of fertilization and varieties factors, for each milling quality and grain and groat size and shape parameters. To complete the analysis, the bimodality of the grain and groat size dimensions was determined. Grain size parameters were considered mixture of two normal distributions and a MATLAB script (MathWorks, 2013) was used to find the maximum likelihood estimation of means and variances of each distribution (see chapter 2 for more details).

4.3 Results

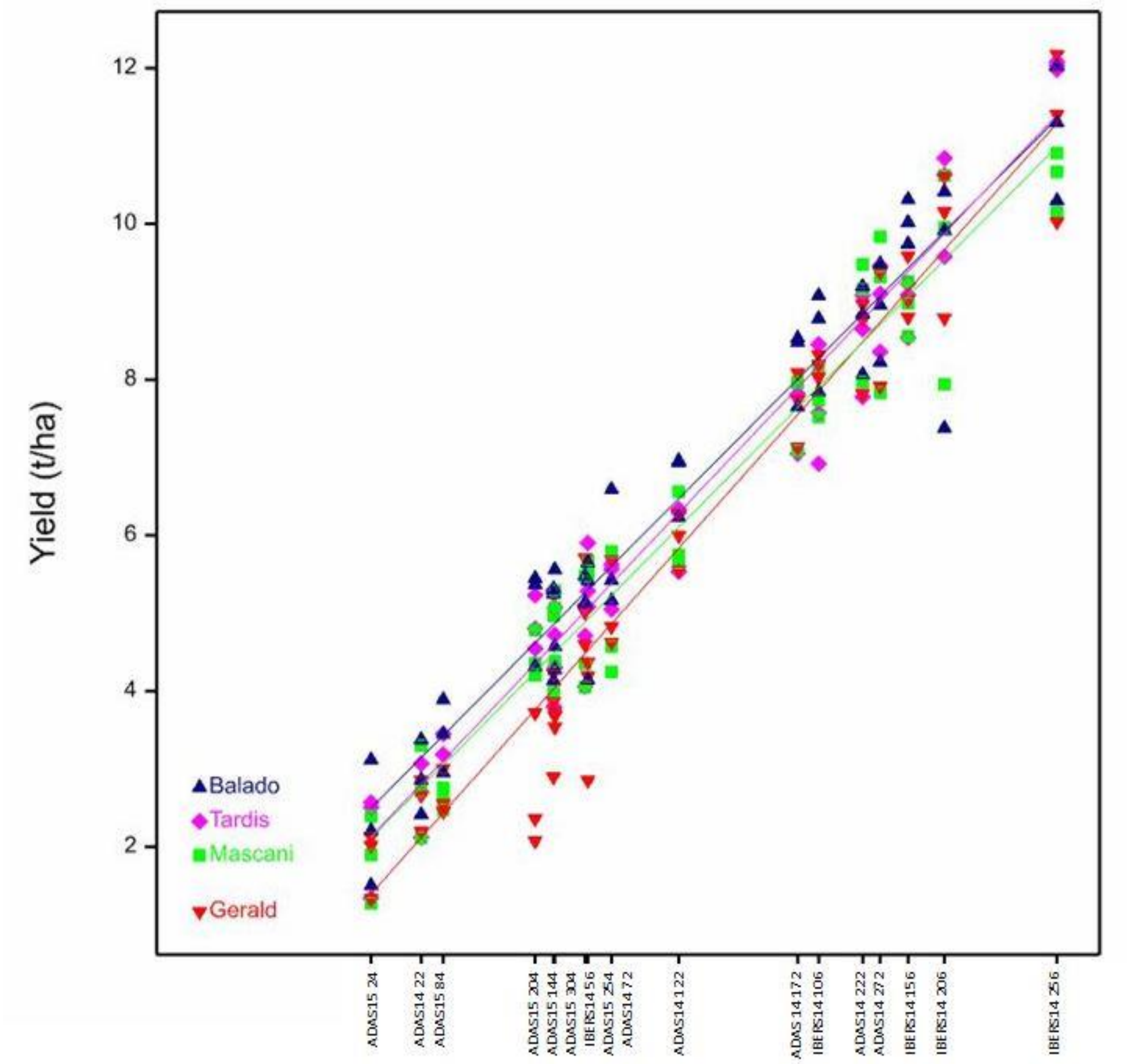
4.3.1 Yield

In all three response experiments, mean yield (t/ha) (figure 4.2) was statistically significant (p-value<0.001) between genotypes, between nitrogen levels of fertilization (p-value <0.001) but there was no significant interaction between the two factors. Joint regression analysis (Finlay & Wilkinson, 1963), was also used to determine phenotypic stability and the sensitivity of trait performance to nitrogen levels (figure 4.2a, figure 4.2.b). In this analysis, the variety performance is plotted against total nitrogen levels mean at each site and a linear regression is performed. This regression of the genotypic response on a total nitrogen level index, such as the average of all phenotypes in a total nitrogen level, is defined as the difference between the marginal mean of the total nitrogen level and the overall mean. The slope of the regression line represents the sensitivity of a variety to the total nitrogen level.



Figures 4.2. *Mean yield (t/ha) ± s.e.m. by nitrogen level of fertilization applied (table 4.2 a, b) at ADAS 2014 and 2015 and IBERS 2014 along with fitted linear regression lines between level of N applied and yield with correlation coefficients.*

In general terms, yield (t/ha) showed a positive and strong relation with increasing levels of fertilization (figure 4.2). The IBERS2014 trial presented the highest yield overall mean, with an increased yield performance of 130% at level 5 of nitrogen applied (250 kg/ha) or total available nitrogen (272 kg/ha) when compared to the no added nitrogen control. At ADAS14, although the mean yield at the highest nitrogen treatment was lower (8.90 t/ha), when compared to level 0 (2.70 t/ha) this represent an increase of yield of 230%. ADAS15 despite having higher maximal levels of nitrogen, applied and in total, i.e. plus the N present in the soil, did not display the same effect with an increase of 123.8% in comparison with level 0.



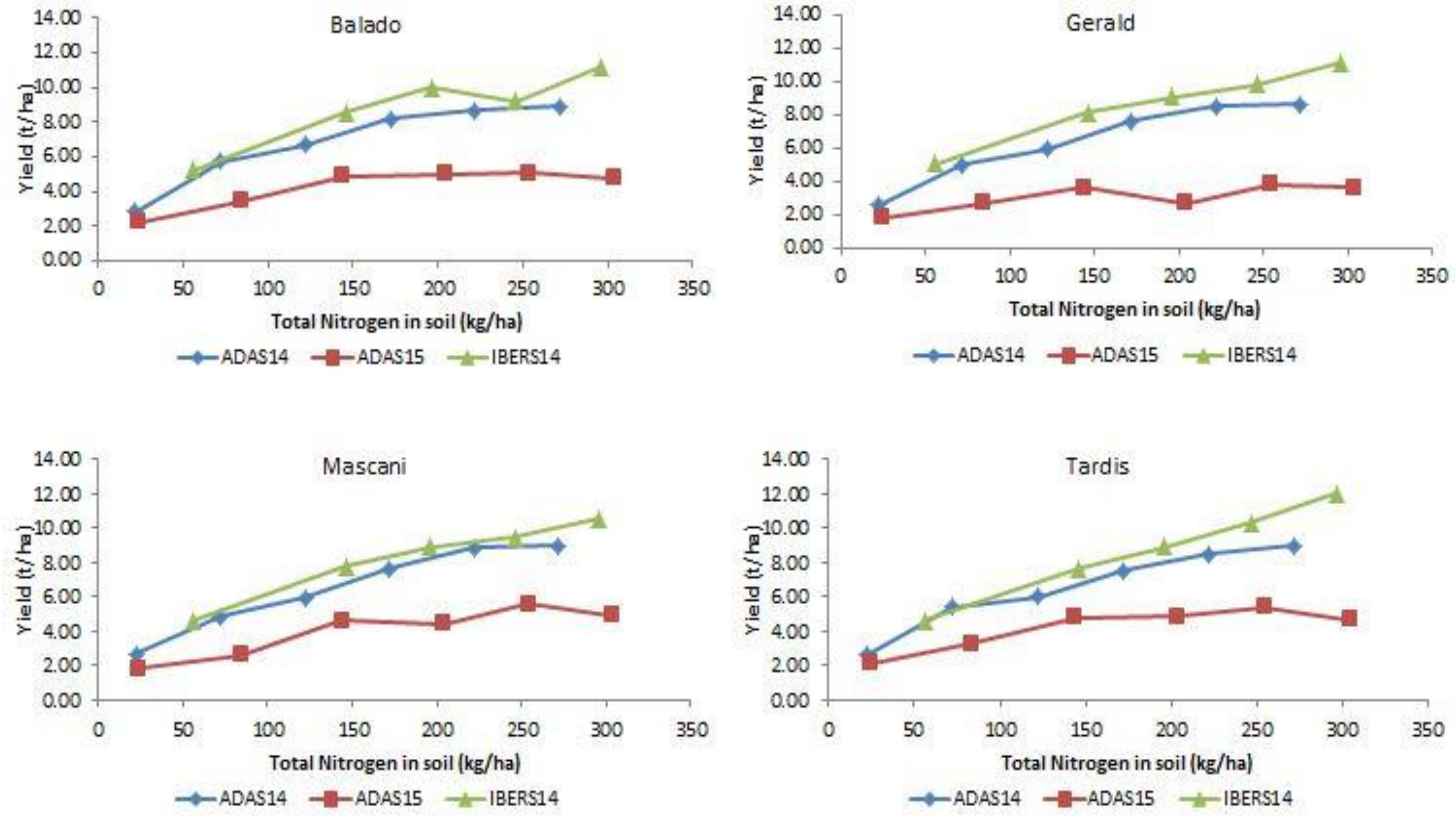
Figures 4.2.b *Joint regression graph for yield (t/ha) of four winter oat varieties and total nitrogen at ADAS14, IBERS14 and ADAS15. 2013- 2014 and 2014-2015 harvest seasons*.

Joint regression analysis (figure 4.2.b and table 4.3.a) showed no statistically significant differences in sensitivity values, but there were between genotypes and total nitrogen levels (p-value<0.001). IBERS14 256 kg/ha, had the higher yield, 11.26 t/ha, whilst ADAS15 24 kg/ha, had the lower, 2.04 t/ha (table 4.3.a).

Table 4.3.a *Mean yield (t/ha) ± s.e.m., and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Yield (t/ha)	s.e.m.	Rank
IBERS 2014	256	11.26	0.237	1
IBERS 2014	206	9.75	0.326	2
IBERS 2014	156	9.26	0.161	3
ADAS 2014	272	8.88	0.201	4
ADAS 2014	222	8.65	0.170	5
IBERS 2014	106	8.05	0.169	6
ADAS 2014	172	7.77	0.142	7
ADAS 2014	122	6.17	0.142	8
ADAS 2014	72	5.27	0.187	9
ADAS 2015	254	4.95	0.259	10
IBERS 2014	56	4.92	0.169	11
ADAS 2015	304	4.51	0.190	12
ADAS 2015	144	4.49	0.228	13
ADAS 2015	204	4.24	0.313	14
ADAS 2015	84	3.01	0.134	15
ADAS 2014	22	2.71	0.123	16
ADAS 2015	24	2.04	0.167	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*Non-significant*	*Non-significant*		*Non-significant*

When analysed by varieties, there was a statistically (p-value<0.001) significant difference between varieties and nitrogen level but no significant interaction between the two factors (table 4.3.b and figure 4.3). Tardis displayed the highest increase in yield in response to N with a 241.03% (8.97 t/ha), at level 5 (250 kg/ha), in comparison with the level 0 (0 kg/ha) value.



Figures 4.3. *Mean yield (t/ha) ± s.e.m. by variety at each level of nitrogen applied (table 4.2 a, b) at ADAS 2014 and 2015 and IBERS 2014.*

Table 4.3.b *Mean yield (t/ha) ± s.e.m. at ADAS 2014 and 2015, and IBERS 2014 harvest season, by variety at each level of nitrogen applied (see table 2.2.a, b).*

		ADAS14		***ADAS15***		***IBERS14***	
	Nitrogen	*Yield*	*s.e.m*	*Yield*	*s.e.m*	*Yield*	*s.e.m*
Balado	0	2.88	0.512	2.28	0.470	6.25	0.120
	1	5.73	0.800	3.43	0.270	10.22	0.384
	2	6.71	0.423	4.90	0.380	11.95	0.171
	3	8.22	0.500	5.05	0.370	11.01	0.970
	4	8.70	0.621	5.07	0.470	13.36	0.516
	5	8.89	0.603	4.80	0.390	0.00	0.000
Gerald	0	2.57	0.382	1.83	0.250	6.09	0.336
	1	5.05	0.644	2.67	0.170	9.75	0.087
	2	5.94	0.412	3.66	0.400	10.90	0.242
	3	7.65	0.520	2.72	0.510	11.74	0.564
	4	8.52	0.621	3.81	0.480	13.36	0.649
	5	8.65	1.000	3.65	0.060	0.00	0.000
Mascani	0	2.71	0.661	1.85	0.320	5.52	0.448
	1	4.87	0.804	2.64	0.080	9.30	0.192
	2	5.99	0.506	4.65	0.340	10.65	0.211
	3	7.67	0.511	4.45	0.170	11.33	0.832
	4	8.87	0.879	5.57	0.050	12.61	0.233
	5	8.99	1.000	4.92	0.270	0.00	0.000
Tardis	0	2.63	0.562	2.14	0.400	5.51	0.315
	1	5.41	0.389	3.32	0.110	9.12	0.458
	2	6.05	0.510	4.78	0.490	10.67	0.219
	3	7.55	0.468	4.86	0.200	12.34	0.403
	4	8.50	0.723	5.42	0.250	14.34	0.029
	5	8.97	0.668	4.70	0.220	0.00	0.000
Significance (G)		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*	
Significance (N)		*p-value<0.001*		*p-value<0.001*		*p-value<0.001*	
Significance (GxN)		*Non-significant*		*Non-significant*		*Non-significant*	

4.3.2 Specific Weight

In all three experiments, there were statistically significant differences in mean specific weight values (kg/hl) between varieties and levels of nitrogen fertilizer and also an interaction between both factors in all sites except IBERS 2014. Specific weight increased at ADAS 2014 and 2015 showed higher values with increasing levels of nitrogen (figure 4.4.a) until reaching the maximum level at level 3 at ADAS 2014, 52.0 kg/hl, and at level 2 at ADAS 2015. At IBERS 2014, specific weight decreased with increasing levels of nitrogen.

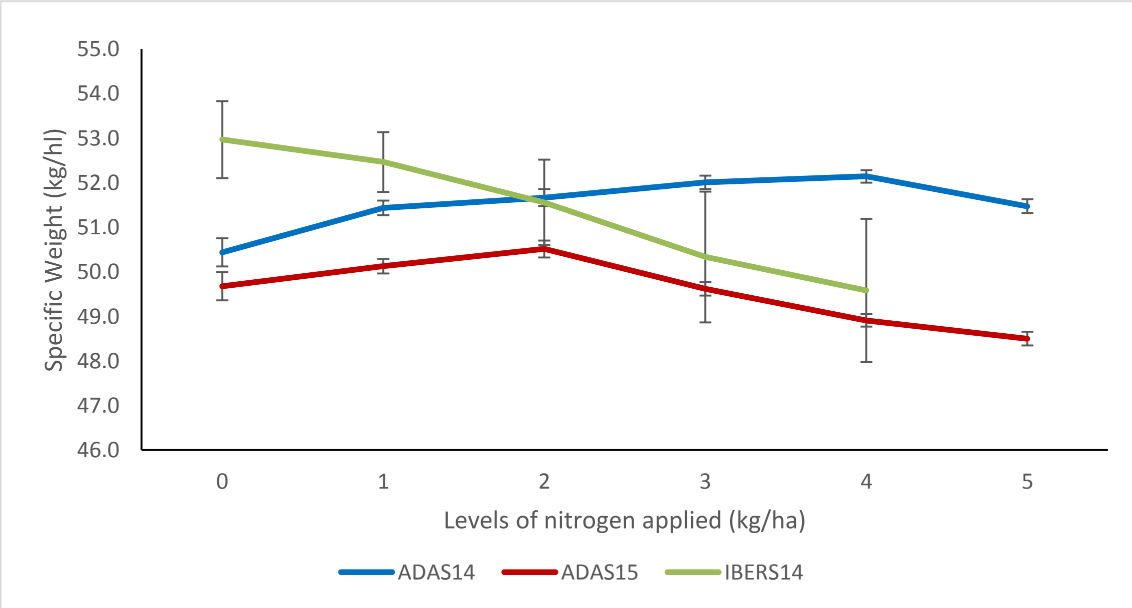


Figure 4.4.a *Mean specific weight (kg/hl) ± s.e.m by nitrogen level of fertilization applied at ADAS 2014 and 2015 and IBERS 2014.*

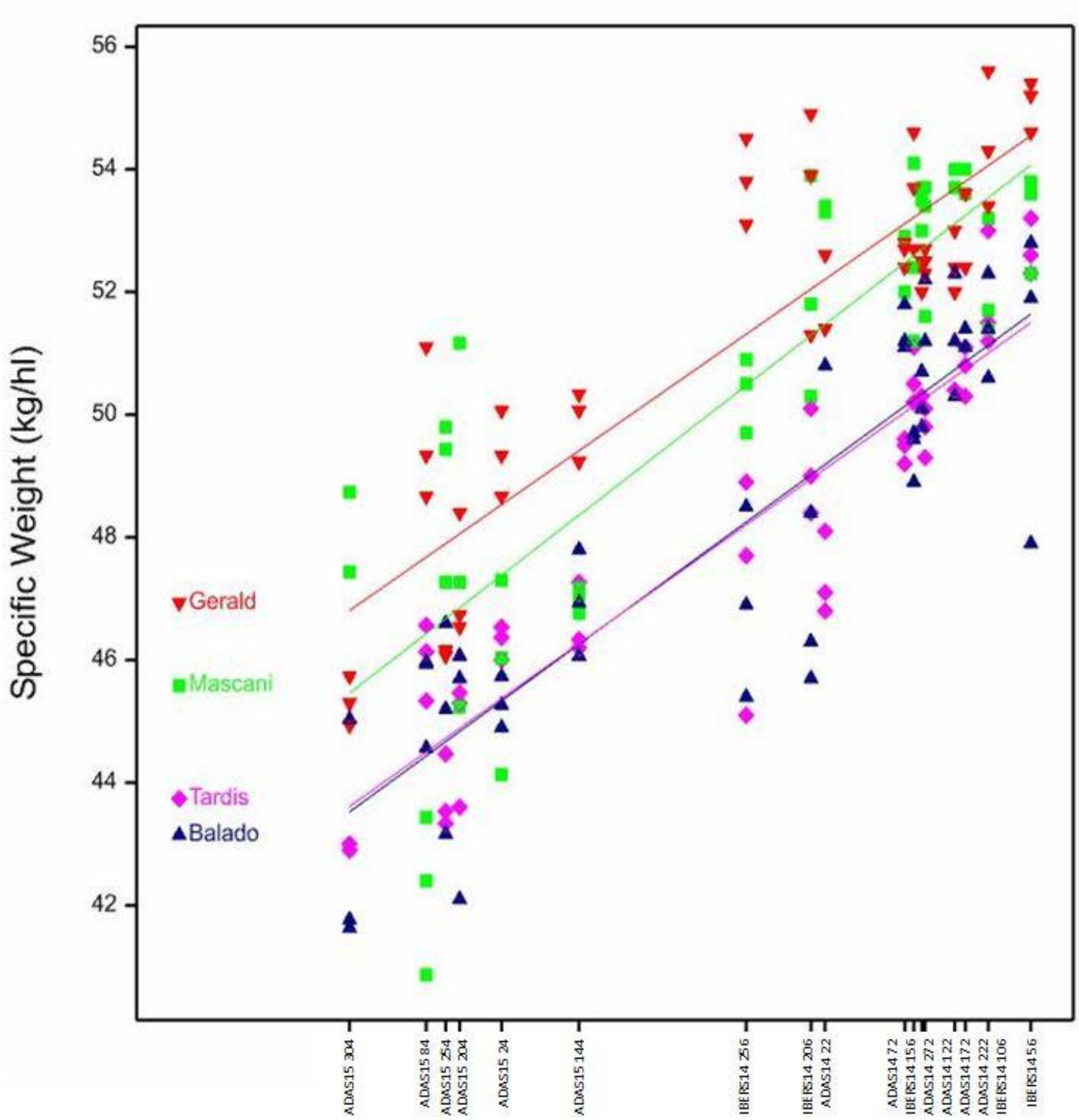


Figure 4.4.b *Joint regression graph for specific weight (kg/hl) for the four winter oat varieties and total nitrogen at ADAS14, IBERS14 and ADAS15. 2013- 2014 and 2014-2015 harvest seasons*.

Joint regression analysis (figure 4.4.b and table 4.5) showed no statistically significant differences in sensitivity values, but there were between genotypes and total nitrogen levels (p-value<0.001). IBERS14 56 kg/ha total nitrogen, showed 52.94 kg/hl mean specific weight whilst ADAS15 304 kg/ha total nitrogen, had 44.85 kg/hl specific weight.

Table 4.5 *Mean specific weight (kg/hl) ± s.e.m. at ADAS 2014 and 2015, and IBERS 2014 harvest season, by variety at each level of nitrogen applied (see table 2.2.a, b).*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Specific Weight (kg/hl)	s.e.m.	Rank
IBERS 2014	56	52.94	0.568	1
IBERS 2014	106	52.44	0.425	2
ADAS 2014	222	52.16	0.374	3
ADAS 2014	172	52.04	0.421	4
ADAS 2014	122	51.68	0.404	5
ADAS 2014	272	51.65	0.430	6
IBERS 2014	156	51.55	0.550	7
ADAS 2014	72	51.44	0.385	8
ADAS 2014	22	50.50	0.791	9
IBERS 2014	206	50.33	0.855	10
IBERS 2014	256	49.56	0.900	11
ADAS 2015	144	47.57	0.426	12
ADAS 2015	24	46.65	0.525	13
ADAS 2015	204	46.15	0.658	14
ADAS 2015	254	45.99	0.630	15
ADAS 2015	84	45.76	0.838	16
ADAS 2015	304	44.85	0.686	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*Non-significant*	*Non-significant*		*Non-significant*

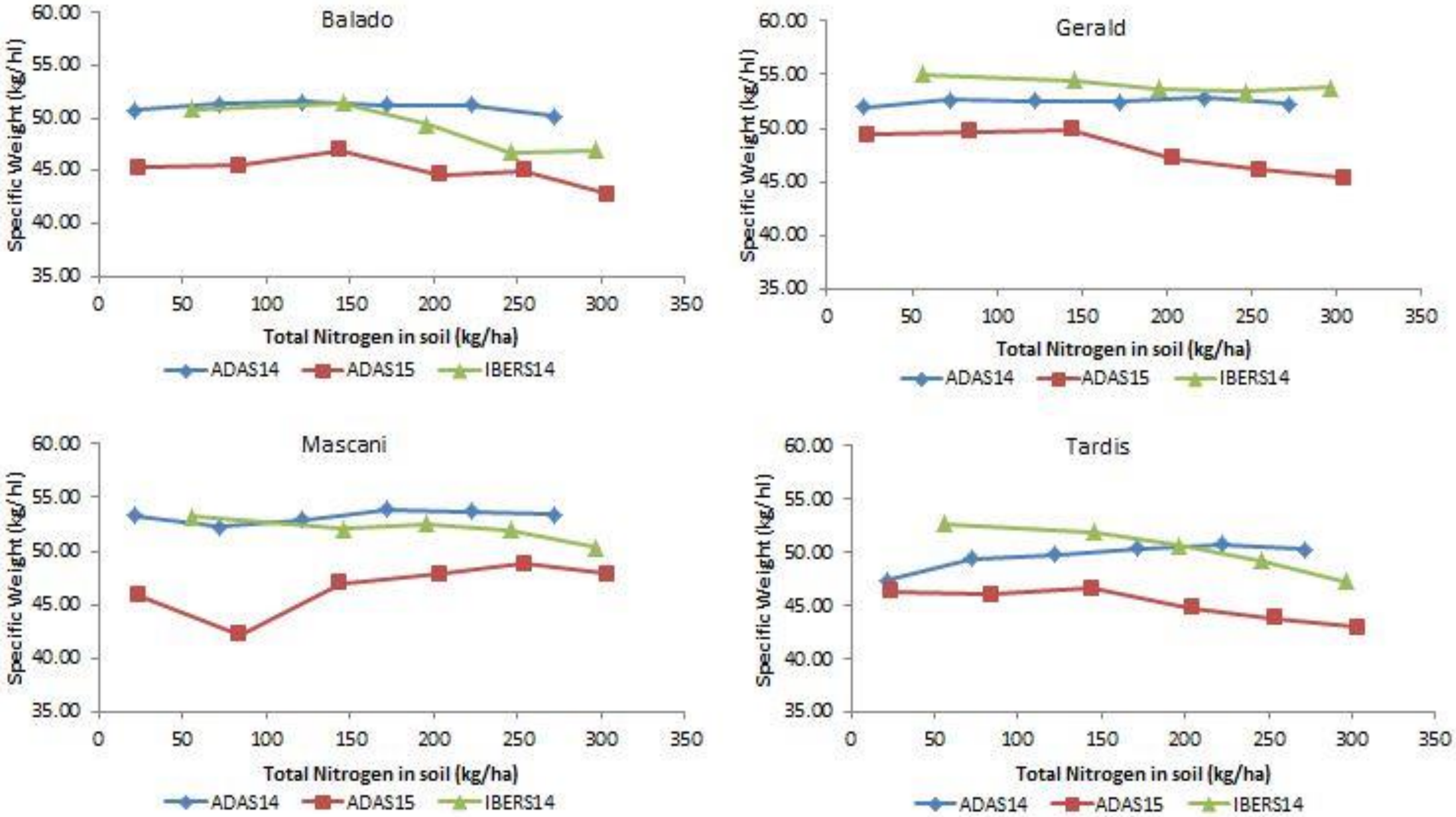


Figure 4.5. *Mean specific weight (t/hl) ± s.e.m by nitrogen level of fertilization applied at ADAS 2014 and 2015, and IBERS 2014.*

By varieties, figure 4.5, there were statistically significant differences (p-value<0.001) in specific weight among varieties and with nitrogen level. There was a significant interaction between variety and nitrogen for ADAS 2015 and ADAS 2014 but not for IBERS 2014.

There was a general trend that increasing levels of nitrogen had a negative effect on specific weight for all varieties with the lowest specific weight obtained at the highest nitrogen treatment. However, for Tardis at ADAS 2014 and Mascani at ADAS 2015, increasing levels of nitrogen resulted in higher specific weight (table 4.5.b, figure 4.5). Gerald and Mascani at ADAS 2014 had relatively stable values for specific weight across all nitrogen treatments (table 4.5.b).

4.3.3 Kernel content

In all three response experiments, mean kernel content (%) (table 4.6.a) showed statistically significant differences (p-value<0.001), between varieties, nitrogen level of fertilization (p-value <0.001) and a significant interaction between these two factors.

Mean kernel content, figure 4.6.a, increased with higher levels of nitrogen applied. Thus, at level 0, ADAS 2014, ADAS 2015 and IBERS 2014 there was an increase of 4.7%, 3.9% and 3.5% respectively between the level 0 value for kernel content in comparison with the highest level of nitrogen applied.

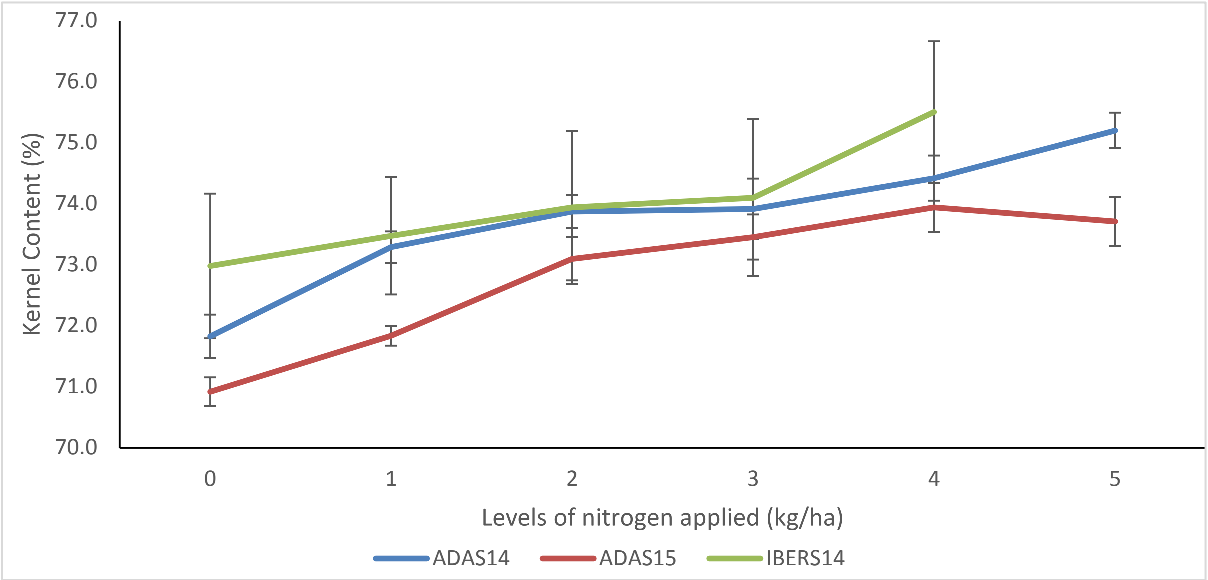


Figure 4.6.a. Average ± s.e.m. kernel content (%) by increasing levels of nitrogen applied (kg/ha) at ADAS 2014, 2015 and Gogerddan 2014 harvest season.

All varieties showed significant differences (p-value<0.001) in kernel content (%) values with increasing levels of nitrogen fertilization applied (table 4.6.b). Tardis presented the highest increase at ADAS 2014, with a 7.32% rise at level 3 (150 kg/ha), 74.72%, with respect to the 0 level of fertilization, 69.62%, whilst Mascani displayed a 7.7% increased kernel content at level 4 in comparison with level 0 at ADAS 2015. This effect was not so high at IBERS 2014 in comparison with the other two sites.

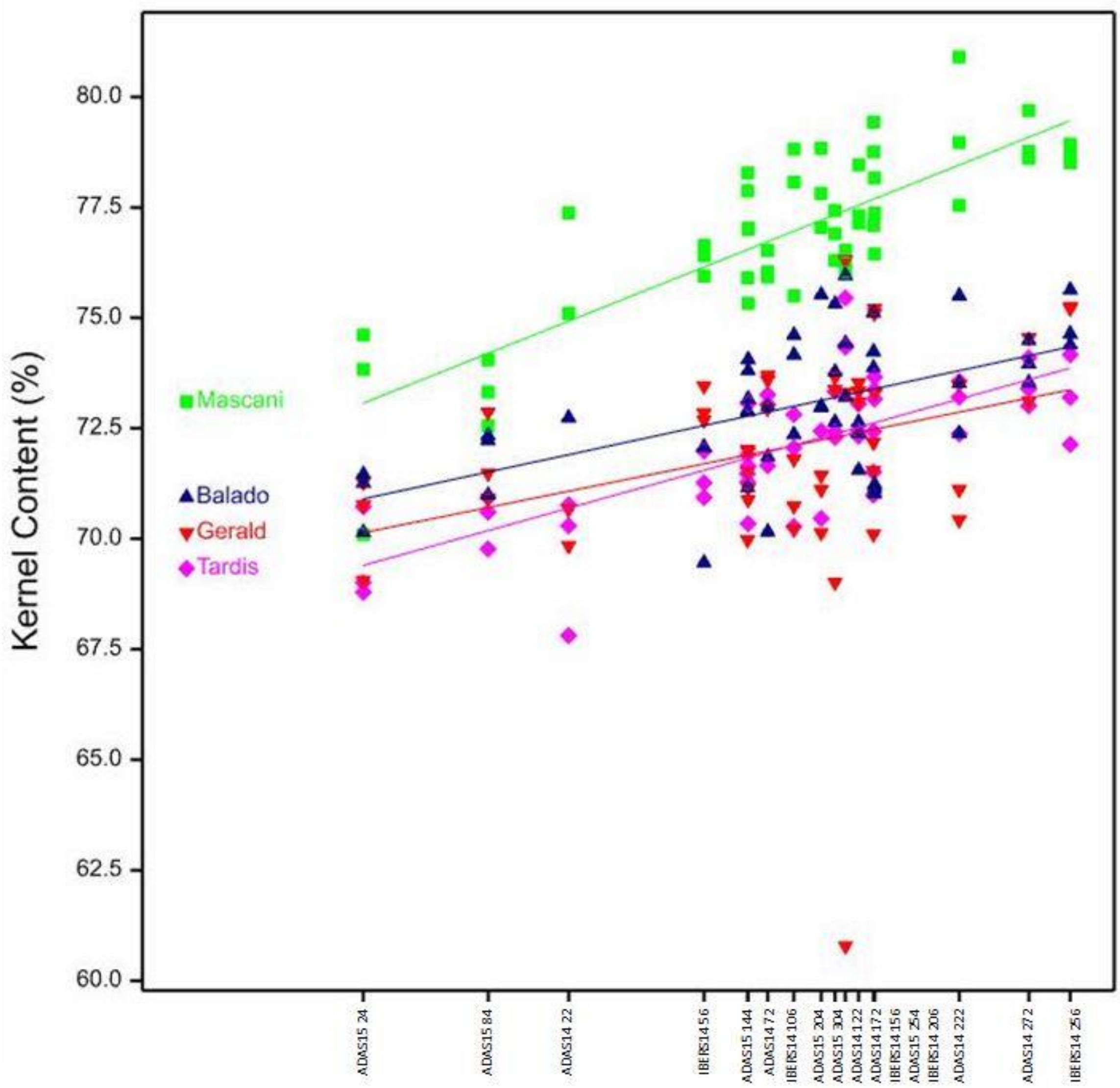


Figure 4.6.b *Joint regression graph for kernel content (%) of four winter oat varieties and total nitrogen at ADAS14, IBERS14 and ADAS15. 2013- 2014 and 2014-2015 harvest seasons*.

Joint regression analysis (figure 4.6.b and table 4.6.a) showed no statistically significant differences in sensitivity values, but there were significant differences between genotypes and total nitrogen levels (p-value<0.001). IBERS14 256 kg/ha N fertilizer had higher kernel content 75.29%, whilst ADAS15 24 kg/ha total nitrogen had the lower kernel content, 70.84% (table 4.6.a).

Table 4.6.a *Mean kernel content (%) ± s.e.m. at ADAS 2014 and 2015, and IBERS 2014 harvest season, by variety at each level of nitrogen applied (see table 2.2.a, b).*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Kernel content (%)	s.e.m.	Rank
IBERS 2014	256	75.29	0.625	1
ADAS 2014	272	75.03	0.727	2
ADAS 2014	222	74.59	0.924	3
IBERS 2014	206	74.06	0.694	4
ADAS 2015	254	74.05	0.893	5
IBERS 2014	156	73.96	0.667	6
ADAS 2014	172	73.87	1.240	7
ADAS 2014	122	73.81	0.670	8
ADAS 2015	304	73.72	0.892	9
ADAS 2015	204	73.55	0.828	10
IBERS 2014	106	73.38	0.548	11
ADAS 2014	72	73.26	0.654	12
ADAS 2015	144	73.26	0.789	13
IBERS 2014	56	72.98	0.654	14
ADAS 2014	22	72.13	0.895	15
ADAS 2015	84	71.62	0.362	16
ADAS 2015	24	70.84	0.520	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*Non-significant*	*Non-significant*		*Non-significant*

Table 4.6.b *Mean kernel content (%) ± s.e.m. at ADAS 2014 and 2015, and IBERS 2014 harvest season, by variety and nitrogen level (see table 2.2.a, b) applied. % refers to the change between the respective 0 level for each site and variety and the nitrogen level applied.*

		ADAS14			***ADAS15***			***IBERS14***		
	Nitrogen Level	***Kernel Content***	***s.e.m***	***% Increase***	***Kernel Content***	***s.e.m***	***% Increase***	***Kernel Content***	***s.e.m***	***% Increase***
Balado	0	72.74	0.000	0.0	70.96	0.091	0.0	71.20	0.195	0.0
	1	73.68	0.264	1.3	71.85	0.096	1.3	71.67	0.184	0.7
	2	73.91	0.775	1.6	72.31	0.129	1.9	72.19	0.073	1.4
	3	74.53	0.795	2.5	73.71	0.153	3.9	71.13	0.013	0.1
	4	73.80	0.909	1.5	74.41	0.082	4.9	74.89	0.085	5.2
	5	74.00	0.275	0.4	73.83	0.189	4.1			
Gerald	0	70.25	0.337	0.0	70.36	0.150	0.0	72.99	0.052	0.0
	1	71.33	0.328	1.5	71.75	0.129	2.0	73.41	0.052	0.6
	2	72.02	1.504	2.5	71.17	0.138	1.1	73.32	0.028	0.4
	3	70.13	4.754	0.2	70.92	0.105	0.8	74.53	0.139	2.1
	4	71.68	0.929	2.0	71.27	0.137	1.3	75.24	0.002	3.1
	5	73.83	0.589	5.1	70.89	0.087	0.8			
Mascani	0	76.24	0.928	0.0	72.85	0.312	0.0	76.33	0.045	0.0
	1	76.45	0.561	0.3	73.31	0.096	0.6	76.16	0.041	0.2
	2	76.88	0.326	0.8	77.35	0.163	6.2	77.64	0.093	1.7
	3	76.27	0.141	0.0	77.46	0.225	6.3	77.33	0.111	1.3
	4	79.14	0.975	3.8	78.42	0.156	7.7	78.71	0.027	3.1
	5	79.02	0.339	3.6	77.90	0.116	6.9			
Tardis	0	69.62	0.918	0.0	69.51	0.137	0.0	71.40	0.069	0.0
	1	71.68	0.785	3.0	70.44	0.077	1.3	72.65	0.113	1.8
	2	72.69	0.328	4.4	71.55	0.040	2.9	72.60	0.051	1.7
	3	74.72	0.363	7.3	71.72	0.168	3.2	73.40	0.033	2.8
	4	73.05	0.352	4.9	71.64	0.094	3.1	73.17	0.131	2.5
	5	73.50	0.318	5.6	71.45	0.181	2.8			
Significance (G)			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (N)			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (GxN)			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	

4.3.4 Thousand Grain Weight

In all three experiments, mean thousand grain weight (g) (figure 4.7.a), showed statistically significant differences between varieties (p-value <0.001), levels of fertilization applied (p-value<0.001) and for the interaction between the two factors (p-value<0.001). There was no consistent effect across the 3 sites studied. At ADAS 2015, there was a general trend of decreasing TGW with application of nitrogen whereas at the other 2 sites, the lowest mean TGW values were obtained at the 0 level of applied nitrogen and varying results obtained at the other levels of N applied.

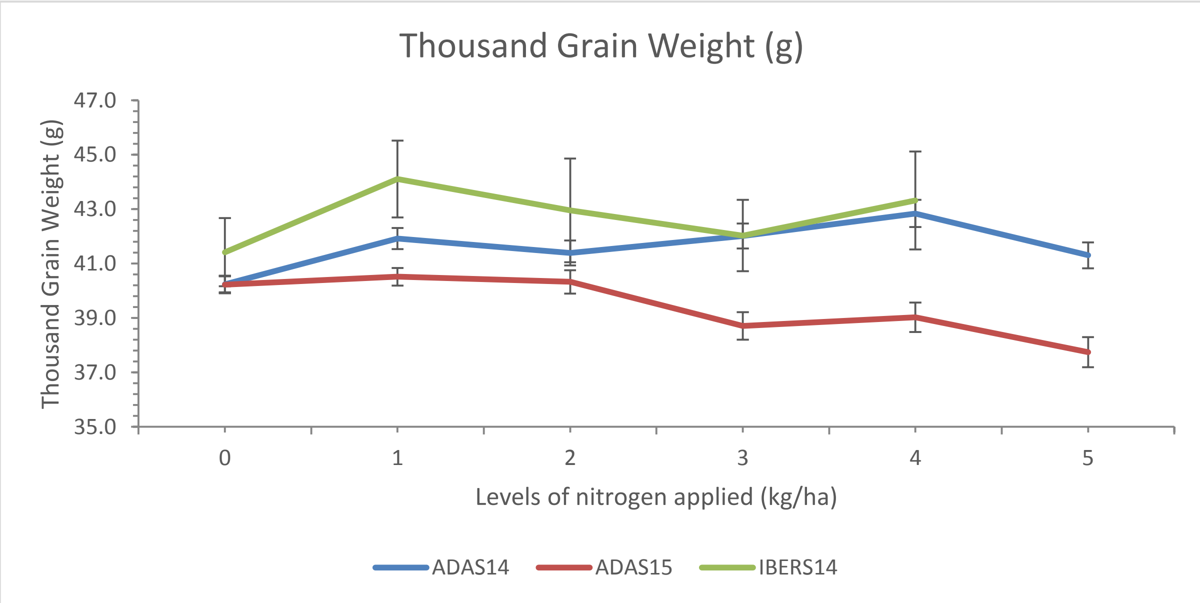


Figure 4.7.a Average ± s.e.m. thousand grain weight (g) value by increasing levels of nitrogen applied (kg/ha) at ADAS 2014 and 2015, and at IBERS 2014 harvest season.

For example, thousand grain weight mean values at ADAS 2014, figure 4.7, increased by 5.7% at level 4 (200 kg/ha) with to respect level 0. However, this increase was not maintained at the highest level, 250 kg /ha. At ADAS 2015 the highest thousand grain weight was found at level 5 of nitrogen applied, a 6.2% increase in comparison with level 0. IBERS 2014 displayed the highest mean thousand grain weight values at level 2.

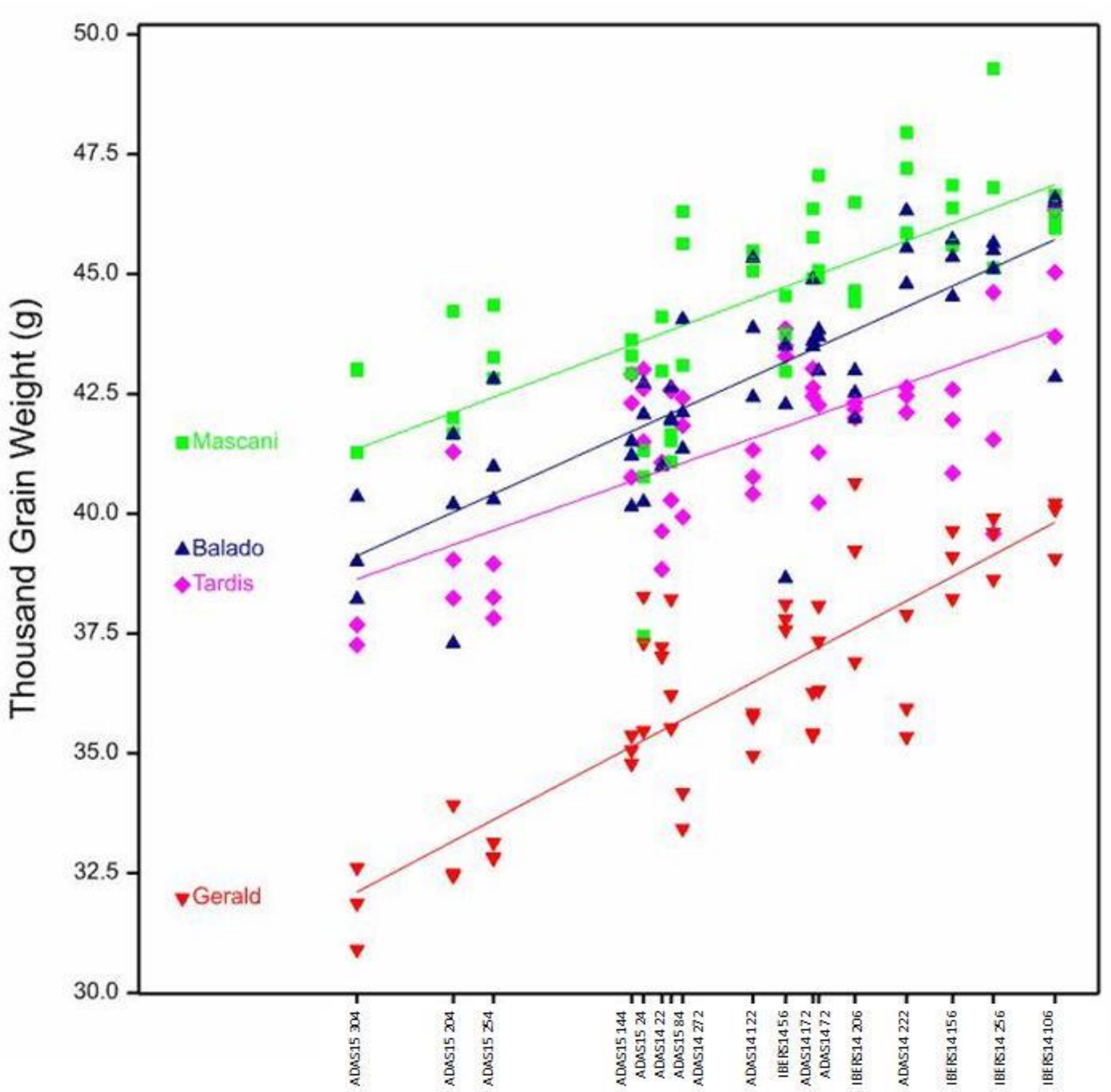


Figure 4.7.b *Joint regression graph for thousand grain weight (g) of four winter oat varieties and total nitrogen at ADAS14, IBERS14 and ADAS15. 2013- 2014 and 2014-2015 harvest seasons*.

Joint regression analysis (figure 4.7.b) showed no statistically significant differences in sensitivity values, but there were between genotypes and total nitrogen levels (p-value<0.001). IBERS14 256 kg/ha total nitrogen showed 37.78 g, whilst IBERS14 106 kg/ total nitrogen had 44.07g (table 4.7.a).

Table 4.7 *Mean thousand grain weight (kg/hl) ± s.e.m. at ADAS 2014 and 2015, and IBERS 2014 harvest season, by variety at each level of nitrogen applied (see table 2.2.a, b).*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Thousand Grain Weight (g)	s.e.m.	Rank
IBERS 2014	106	44.07	0.828	1
IBERS 2014	256	43.52	0.994	2
IBERS 2014	156	43.15	0.885	3
ADAS 2014	222	42.73	1.251	4
IBERS 2014	206	42.27	0.726	5
ADAS 2014	72	41.94	0.969	6
ADAS 2014	172	41.89	1.157	7
IBERS 2014	56	41.64	0.790	8
ADAS 2014	122	41.35	1.150	9
ADAS 2014	272	40.72	1.196	10
ADAS 2015	84	40.61	0.721	11
ADAS 2014	22	40.53	0.736	12
ADAS 2015	24	40.36	0.723	13
ADAS 2015	144	40.26	0.963	14
ADAS 2015	254	39.01	1.123	15
ADAS 2015	204	38.65	1.137	16
ADAS 2015	304	37.78	1.240	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*Non-significant*	*Non-significant*		*Non-significant*

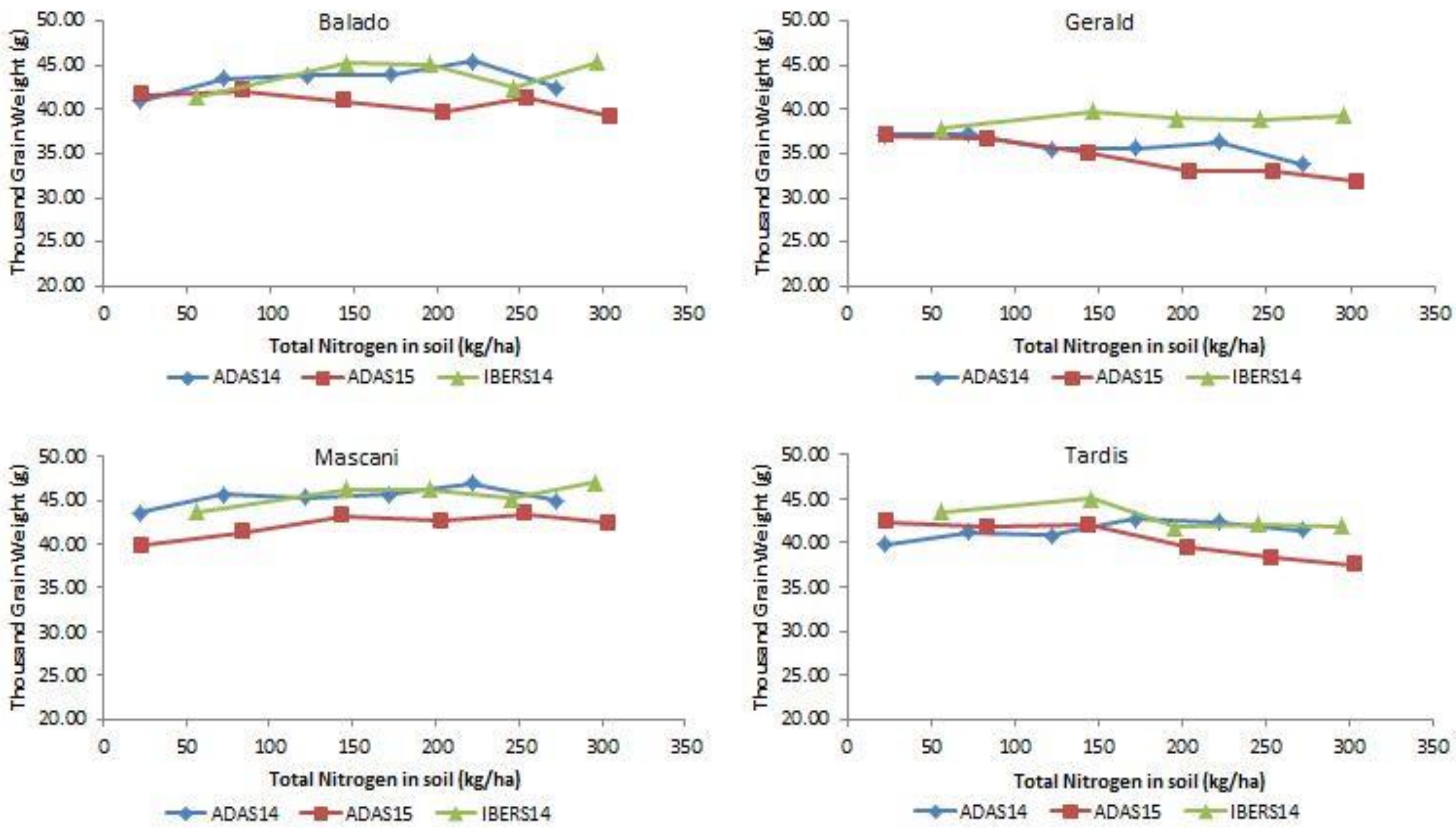


Figure 4.8. Average ± s.e.m. thousand grain weight (g) value by variety and increasing levels of nitrogen applied (kg/ha) at ADAS 2014, 2015 and IBERS 2014.

When the nitrogen response of thousand grain weight values of individual varieties are examined (figure 4.8), no clear trends are apparent. For example, the highest increases in thousand grain weight were found for Balado, which represent an 8.8% rise at level 4 at ADAS 2014 when compared to TGW level 0 of fertilization values at the same site, and a 10.1% higher thousand gran weight at level 4 at IBERS 2014 respect to level 0 of fertilization at IBERS 2014. Such increases in thousand grain weight were not found at ADAS 2015, where all varieties showed lower values with increasing levels of nitrogen applied. Interestingly, Gerald showed the stronger diminishing effect with higher levels of nitrogen applied at ADAS 2015, with a 14.1% lower TGW at level 5 compared with level 0 (table 4.7.b).

4.3.5 Hullability

In all three experiments, mean hullability (%) (table 4.9) showed statistically significant differences (p-value<0.001) between varieties and nitrogen level but only a significant interaction between the two factors at ADAS 2015.

Mean hullability (%) values for nitrogen level at each site, table 4.9 and figure 4.9.a, displayed increased values with increasing levels of nitrogen applied or total, i.e. SMN plus applied, at all sites. ADAS 2014 showed the highest increase with 21% higher results at level 5 in comparison with level 0. This rate of increase was lower at ADAS 2015, 12.5%, and at IBERS 2014, 9%.

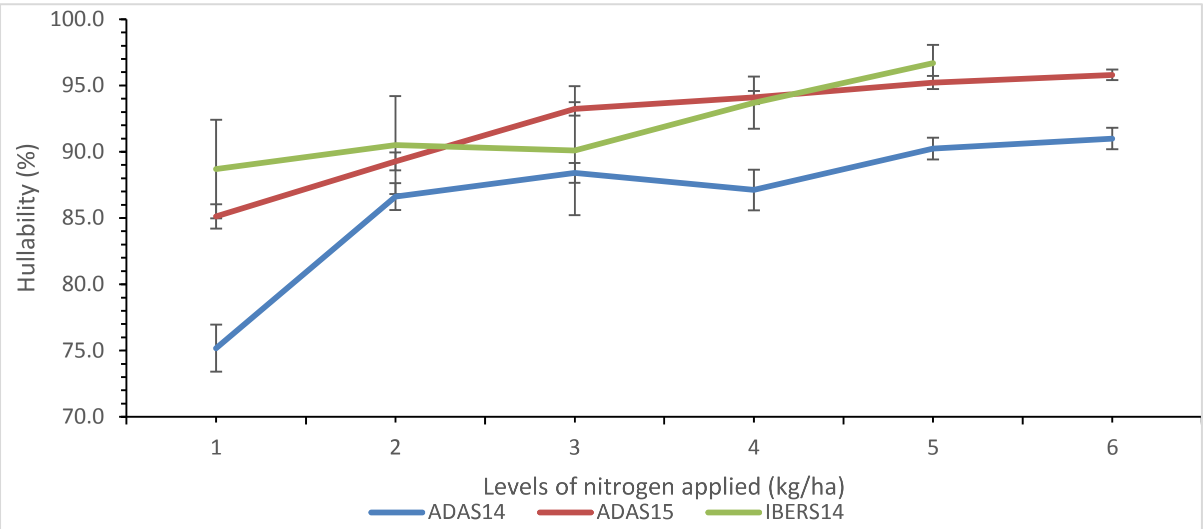


Figure 4.9.a *Mean hullability (%) ± s.e.m. values by increasing levels of nitrogen applied (kg/ha) at ADAS 2014, 2015 and IBERS 2014.*

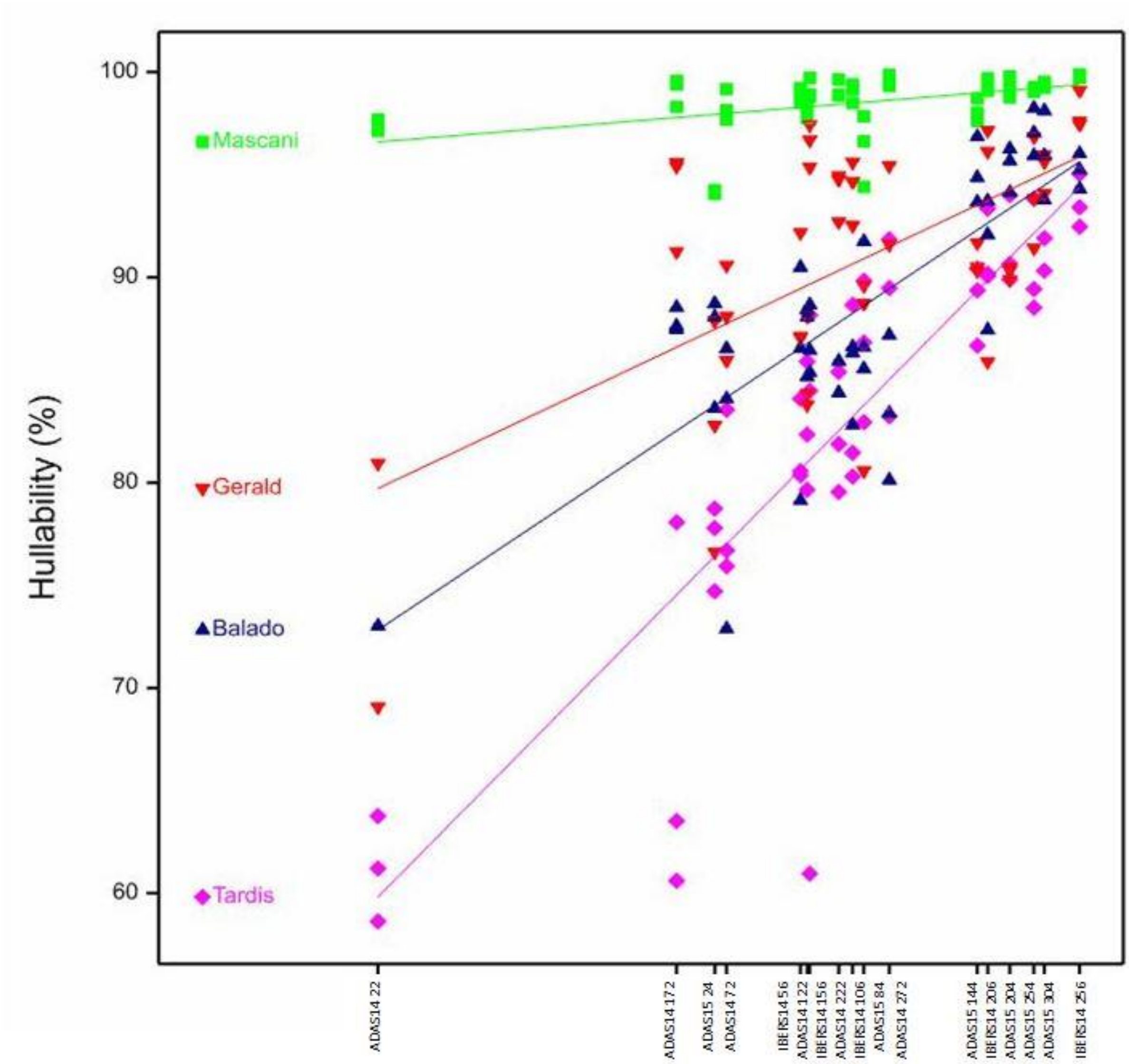


Figure 4.9.b *Joint regression graph for hullability (%) of four winter oat varieties and total nitrogen at ADAS14, IBERS14 and ADAS15. 2013- 2014 and 2014-2015 harvest seasons*.

Joint regression analysis (figure 4.9.b, table 4.9) showed statistically significant differences in sensitivity values, genotypes and total nitrogen levels (p-value<0.001). Mascani showed a lower sensitivity value, 0.14, therefore higher stability against total nitrogen levels whilst Tardis with sensitivity value of 1.721, showed lower stability against changes in total nitrogen levels. IBERS14 206 kg/ha total nitrogen showed higher hullability, 96.7%, than ADAS14 22 kg/ha total nitrogen; 76.6% (table 4.9).

Table 4.9 *Mean hullability (%) ± s.e.m., and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	TOTAL Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Hullability (%)	s.e.m.	Rank
IBERS 2014	256	96.66	0.760	1
ADAS 2015	304	95.65	0.931	2
ADAS 2015	254	95.35	1.097	3
ADAS 2015	204	94.66	1.106	4
IBERS 2014	206	94.02	1.365	5
ADAS 2015	144	93.72	1.143	6
ADAS 2014	272	91.20	2.028	7
ADAS 2015	84	90.48	1.516	8
IBERS 2014	106	90.16	2.035	9
ADAS 2014	222	89.76	2.070	10
IBERS 2014	156	88.93	3.131	11
ADAS 2014	122	88.85	1.872	12
IBERS 2014	56	88.6	2.109	13
ADAS 2014	72	86.54	2.535	14
ADAS 2015	24	86.21	2.055	15
ADAS 2014	172	85.11	3.826	16
ADAS 2014	22	76.56	5.45	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*

All varieties showed increased mean hullability (%) with increasing levels of nitrogen fertilization applied (kg/ha) (figure 4.10). A lower response to nitrogen was found with Mascani, with a maximum effect at ADAS 2015 of a 5.5% increase in hullability at level 5, in comparison with level 0. However, Mascani displayed very high hullability values at the 0 level of applied N at all sites. Balado, Gerald and Tardis, all had relatively low hullability values at 0 level nitrogen at all sites and displayed increases in response to applied nitrogen. For example Tardis had 44.1% higher mean hullability value at level 5 (88.19%) in comparison with level 0 at ADAS 2014 (61.2%) mean hullability value.

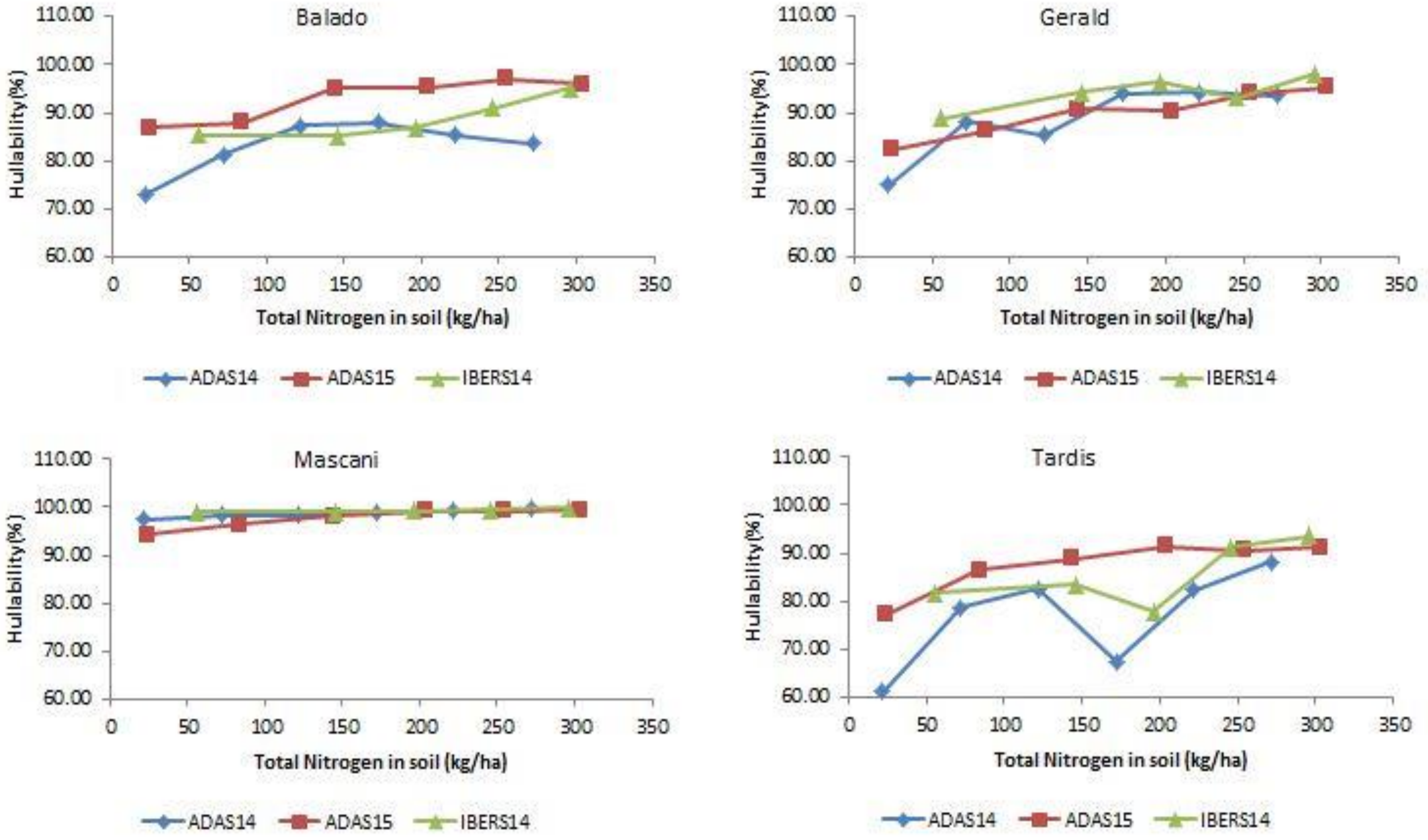


Figure 4.10. *Average ± s.e.m. hullability (%) values by variety and increasing levels of nitrogen applied (kg/ha) at ADAS 2014, 2015 and IBERS 2014*.

4.3.6 Chemical quality traits. Oil, protein and β-glucan content

Chemical quality traits measured included oil, protein and β-glucan content, determined as described in chapter 2, material and methods.

In all three experiments, mean oil content (%) values were statistically significant (p-value<0.001) by two way ANOVA for varieties (figure 4.12) and nitrogen levels (figure 4.11.a) but not significant for the interaction between the two factors (p-value>0.05) for all sites.

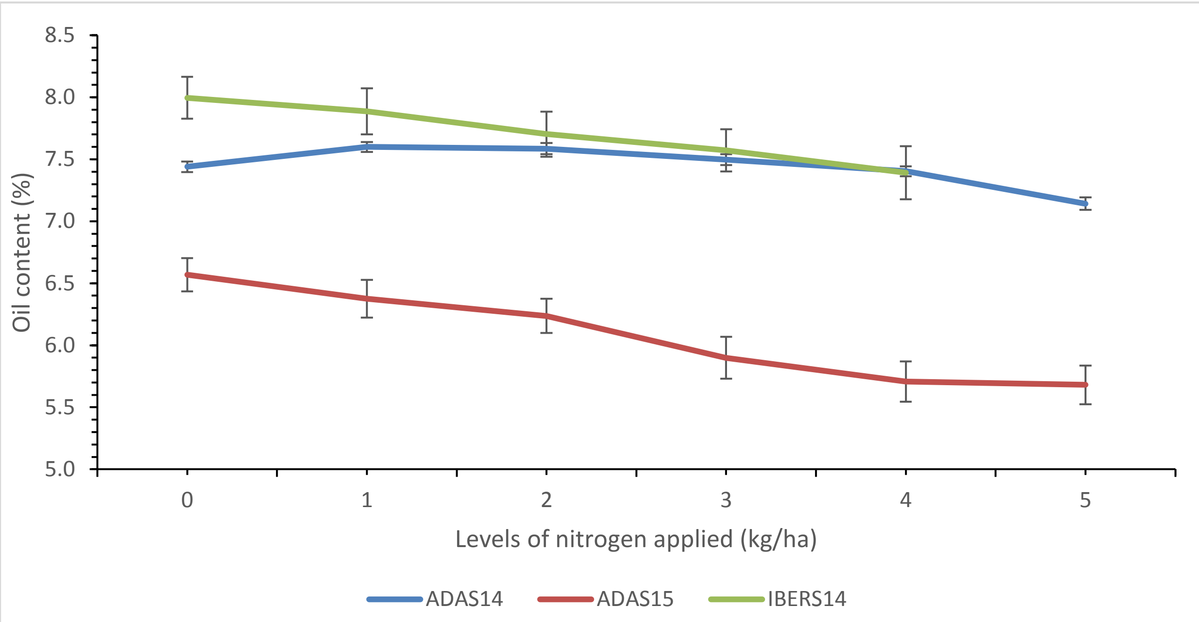


Figure 4.11.a *Mean oil content (%) ± s.e.m values by increasing levels of nitrogen applied (kg/ha) at ADAS 2014, 2015 and IBERS 2014.*

Mean oil content by nitrogen levels and sites displayed lower values (figure 4.11.a) with increasing levels of nitrogen applied or total, i.e. SMN and N applied to soil. This diminishing effect was greater at ADAS 2015, with a 13.5% lower value at level 5 of nitrogen compared with level 0. IBERS 2014 also showed this effect whilst ADAS 2014 only had lower results from level 3 onwards, having higher results at level 1 and 2 when compared with level 0.

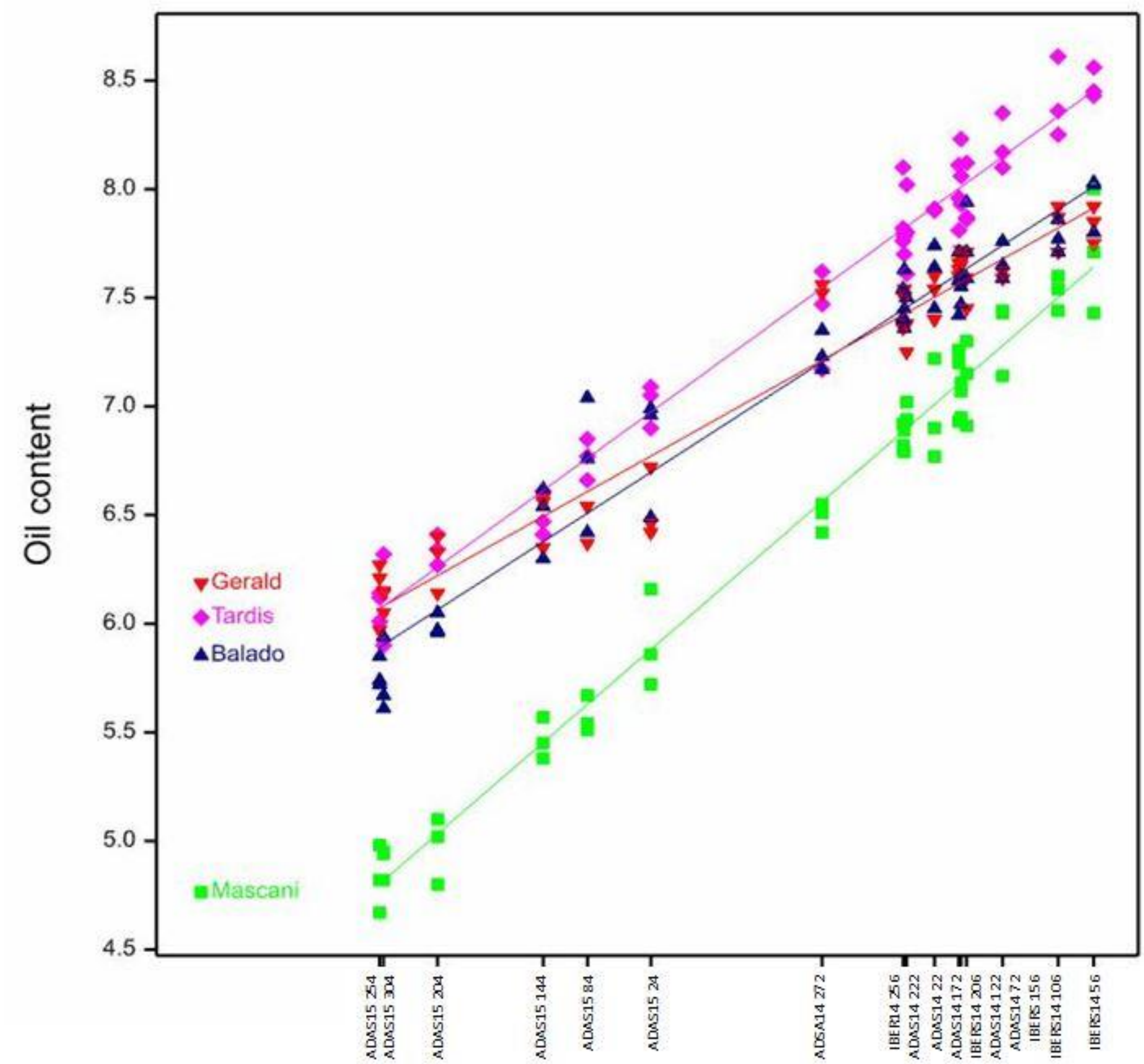


Figure 4.11.b *Joint regression graph for oil content (%) of four winter oat varieties and total nitrogen at ADAS14, IBERS14 and ADAS15. 2013- 2014 and 2014-2015 harvest seasons*.

Joint regression analysis (figure 4.11.b. table 4.10.a) showed statistically significant differences in sensitivity values, genotypes and total nitrogen levels (p-value<0.001). Gerald showed a lower sensitivity value, 0.80, and therefore higher stability against changes in total nitrogen levels whilst Mascani with sensitivity value of 1.24, showed lower stability against changes in total nitrogen levels. IBERS14 56 kg/ha total nitrogen showed higher oil content 8.0%, whilst ADAS14 254 kg/ha total nitrogen; showed the lower, 5.7% (table 4.10.a).

Table 4.10.a *Mean oil content (%) ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Oil content (%)	s.e.m.	Rank
IBERS 2014	56	8.01	0.097	1
IBERS 2014	106	7.89	0.101	2
IBERS 2014	156	7.71	0.099	3
ADAS 2014	72	7.60	0.101	4
ADAS 2014	122	7.58	0.114	5
IBERS 2014	206	7.57	0.095	6
ADAS 2014	172	7.49	0.109	7
ADAS 2014	22	7.40	0.131	8
ADAS 2014	222	7.40	0.102	9
IBERS 2014	256	7.39	0.115	10
ADAS 2014	272	7.13	0.445	11
ADAS 2015	24	6.58	0.135	12
ADAS 2015	84	6.38	0.151	13
ADAS 2015	144	6.23	0.138	14
ADAS 2015	204	5.90	0.168	15
ADAS 2015	304	5.72	0.163	16
ADAS 2015	254	5.71	0.163	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*

The effect described above for overall results was also found when the results for each variety are examined. At IBERS 2014 and ADAS 2015, all varieties displayed lower oil contents as the level of nitrogen applied increased. At ADAS 2014 all varieties, table 4.10.b, showed a slight increase in oil content at the lower levels of nitrogen applied, levels 1 (50 kg/ha), 2 (100 kg/ha) and 3 (150 kg/ha), with a final decline in oil content at the highest level of nitrogen applied, 250 kg /ha. Mascani, with a 7% less oil content compared to the level 0, was the one most affected by higher levels of nitrogen applied (table 4.10.b).

Table 4.10.b *Mean oil content (%) ± s.e.m. at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied. % refers to the change between the respective 0 level for each site and variety and the nitrogen level applied.*

		ADAS14			**ADAS15**			**IBERS14**		
	Nitrogen level	***Oil content***	**s.e.m**	***% Increase***	**Oil content**	**s.e.m**	***% Increase***	**Oil content**	**s.e.m**	***% Increase***
Balado	0	7.50	0.000	0.0	6.81	0.162	0.0	7.95	0.017	0.0
	1	7.75	0.103	3.3	6.74	0.179	-1.1	7.78	0.010	-2.1
	2	7.54	0.038	0.5	6.49	0.096	-4.8	7.67	0.011	-3.6
	3	7.61	0.085	1.5	5.99	0.028	-12.0	7.57	0.019	-4.8
	4	7.48	0.079	-0.3	5.77	0.040	15.3	7.45	0.010	-6.3
	5	7.25	0.053	-3.3	5.74	0.101	-15.8			
Gerald	0	7.32	0.053	0.0	6.53	0.094	0.0	7.84	0.011	0.0
	1	7.58	0.075	3.7	6.43	0.057	-1.6	7.83	0.014	-0.1
	2	7.69	0.020	5.1	6.50	0.074	-0.6	7.60	0.002	-3.1
	3	7.51	0.059	2.7	6.29	0.078	-3.7	7.63	0.004	-2.7
	4	7.52	0.012	2.8	6.15	0.092	-5.9	7.37	0.001	-6.0
	5	7.54	0.016	3.1	6.11	0.032	-6.4			
Mascani	0	6.98	0.033	0.0	5.91	0.130	0.0	7.71	0.037	0.0
	1	7.12	0.114	2.0	5.57	0.049	-5.7	7.53	0.010	-2.4
	2	7.04	0.048	0.9	5.47	0.055	-7.6	7.34	0.022	-4.9
	3	6.96	0.134	-0.2	4.97	0.090	-15.9	7.13	0.023	-7.6
	4	6.86	0.033	-1.8	4.82	0.090	-18.4	6.85	0.008	-11.2
	5	6.49	0.038	-7.0	4.90	0.042	-17.1			
Tardis	0	7.81	0.118	0.0	7.01	0.058	0.0	8.48	0.009	0.0
	1	7.95	0.085	1.8	6.76	0.055	-3.6	8.41	0.024	-0.9
	2	8.07	0.087	3.4	6.50	0.059	-7.4	8.20	0.017	-3.2
	3	7.90	0.003	1.2	6.34	0.040	-9.6	7.96	0.019	-6.1
	4	7.76	0.031	-0.6	6.09	0.040	-13.2	7.89	0.023	-6.9
	5	7.42	0.132	-5.0	6.11	0.171	-12.9			
Significance (G)			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (N)			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (GxN)			*Non-significant*			*Non-significant*			*Non-significant*	

In all three experiments, mean protein content displayed statistically significant differences (p-value<0.001) between applied nitrogen levels, when analysed by two way ANOVA (p-value <0.001). However, there were no significant differences between varieties (p-value>0.05) nor an interaction between the two factors (p-value>0.05).

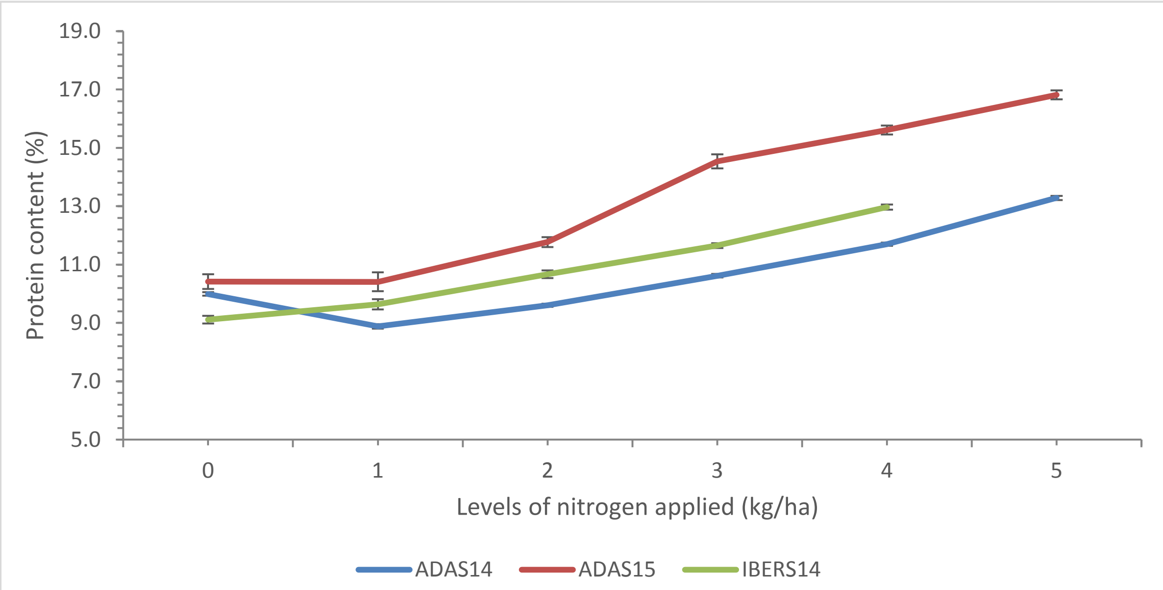


***Figure 4.12.a** Mean protein content (%)* ± s.e.m values by increasing levels of nitrogen applied (kg/ha) at ADAS 2014 and 2015, and at IBERS 2014 harvest seasons.

A mirror effect was found for protein content (%) (figure 4.12.a) when compared with the effect of higher levels of fertilizer found for oil content (%). Thus, all sites showed higher results of protein content with increasing application of nitrogen. For example grain from ADAS 2015 level 5 with a 49.9% more protein content (15.6%), than at level 0 (10.4%); whilst oil content as mentioned above decreased with higher levels of nitrogen (table 4.11.b).

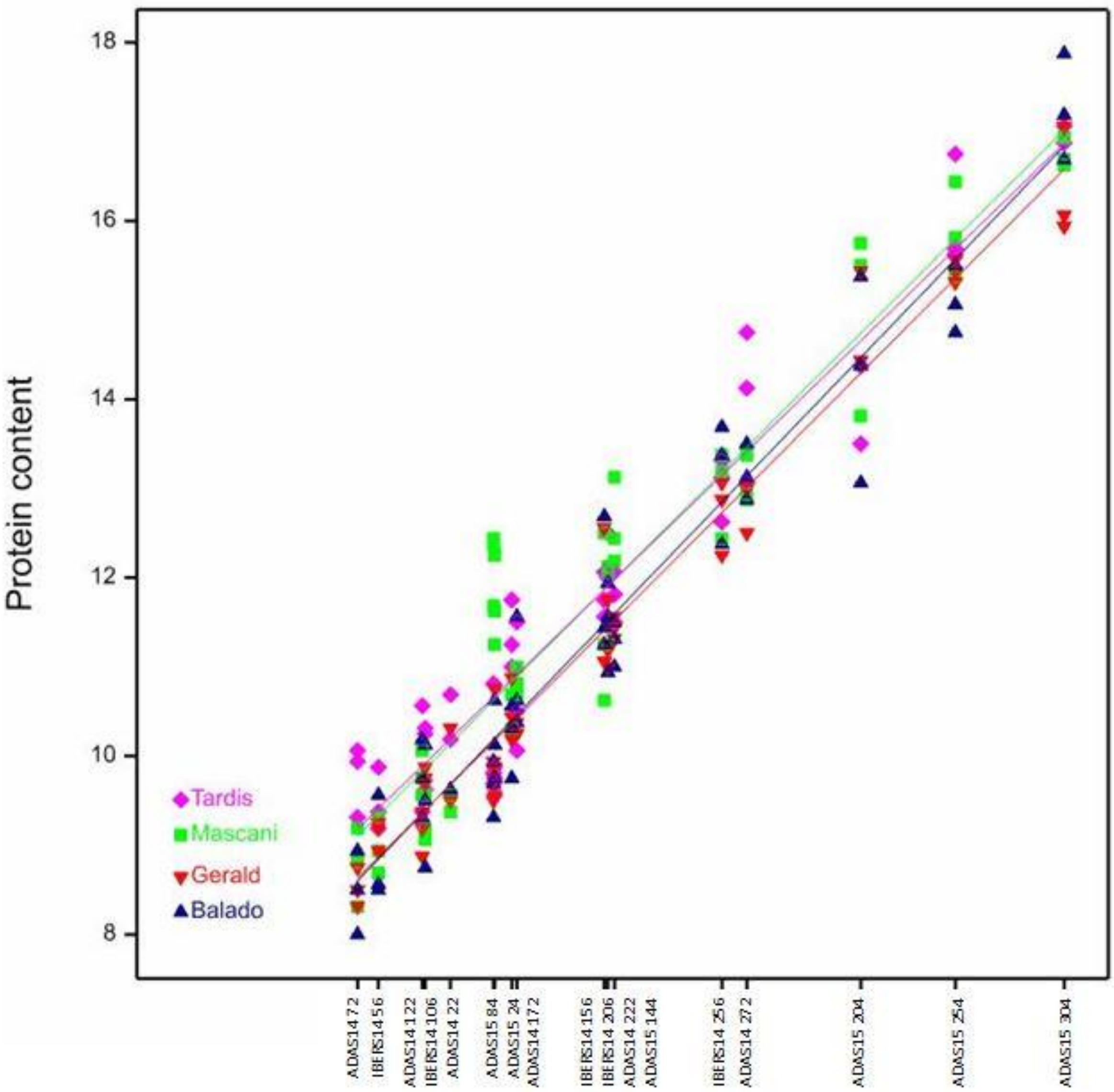


Figure 4.12.b *Joint regression graph for protein content (%) of four winter oat varieties and total nitrogen at ADAS14, IBERS14 and ADAS15. 2013- 2014 and 2014-2015 harvest seasons*.

Joint regression analysis (figure 4.12.b and table 4.11) showed no statistically significant differences in sensitivity values, but there were between total nitrogen levels (p-value<0.001). ADAS15 304 kg/ha total nitrogen had higher protein content, 16.8 (%), whilst ADAS14 72 kg/ha total nitrogen showed 8.9 (%) protein content.

Table 4.11 *Mean protein content (%) ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Protein content (%)	s.e.m.	Rank
ADAS 2015	304	16.83	0.160	1
ADAS 2015	254	15.61	0.157	2
ADAS 2015	204	14.54	0.243	3
ADAS 2014	272	13.26	0.186	4
IBERS 2014	256	12.98	0.133	5
ADAS 2014	144	11.77	0.171	6
ADAS 2014	222	11.7	0.145	7
IBERS 2014	206	11.65	0.193	8
IBERS 2014	156	10.67	0.139	9
ADAS 2014	172	10.61	0.155	10
ADAS 2015	24	10.42	0.257	11
ADAS 2015	84	10.41	0.327	12
ADAS 2014	22	9.92	0.191	13
IBERS 2014	106	9.64	0.137	14
ADAS 2014	122	9.61	0.140	15
IBERS 2014	56	9.11	0.118	16
ADAS 2014	72	8.88	0.185	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*No significant*	*No significant*		*No significant*

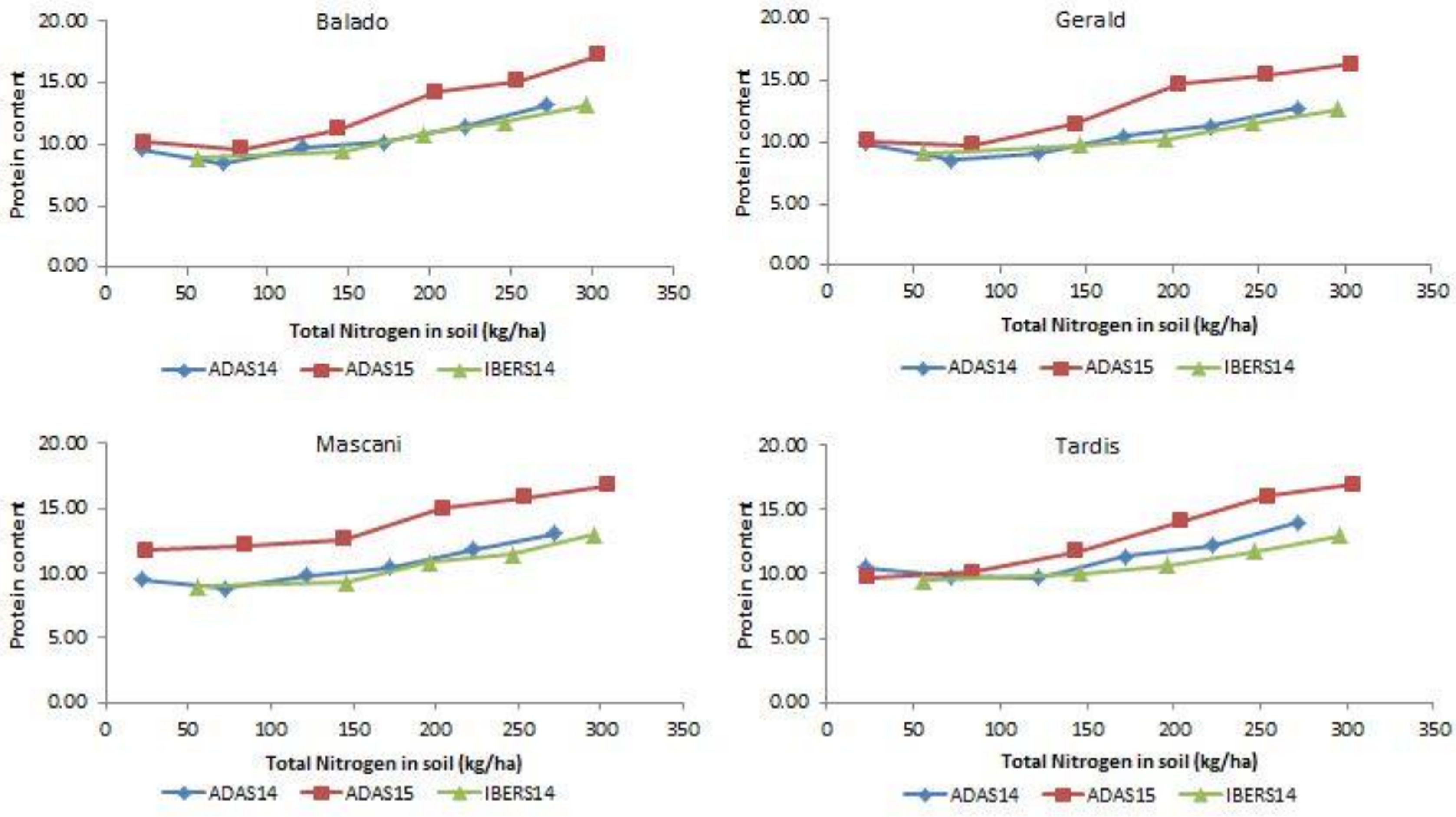


Figure 4.13 *Mean protein content (%) ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at ADAS 2014, 2015 and IBERS 2015, harvest seasons*.

Although non-statistically significant differences were found between varieties (figure 4.13) in protein content (p-value>0.05) there was a positive effect on protein content with higher levels of fertilizer. For example, Tardis had a 74.4% higher protein content at level 5 (17.0%) ADAS 2015 in comparison with a 10.2% protein content at level 0 at that site.

In all three experiments, mean β-glucan content showed statistically significant differences (p-value<0.001) with nitrogen levels by two-way ANOVA (figure 4.14.a). β-glucan content increased with increasing levels of nitrogen fertilizer. Thus, ADAS 2014 showed a 14.9% increase β-glucan content in comparison with level 0. The effect of nitrogen on β-glucan at ADAS 2015 and IBERS 2014 was slightly lower also showed 10.4% and 9.4%, respectively, but still higher β-glucan at level 5 in comparison with level 0.

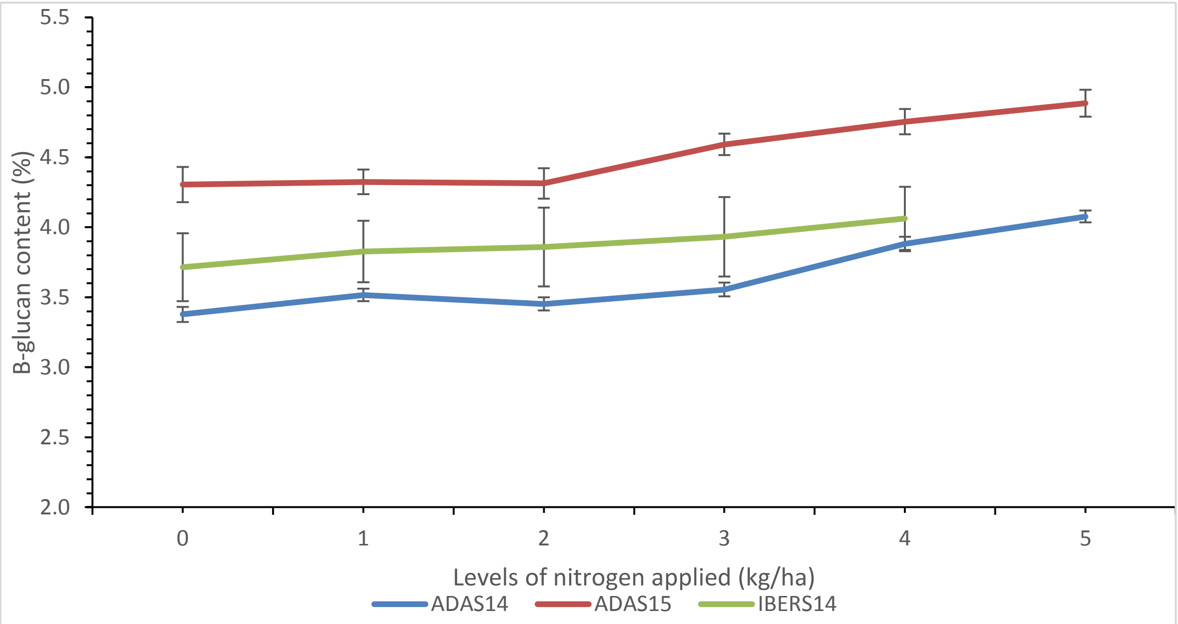


Figure 4.14.a *Mean β-glucan content (%) ± s.e.m values by increasing levels of nitrogen applied (kg/ha) at ADAS 2014 and 2015, and IBERS 2014 harvest season.*

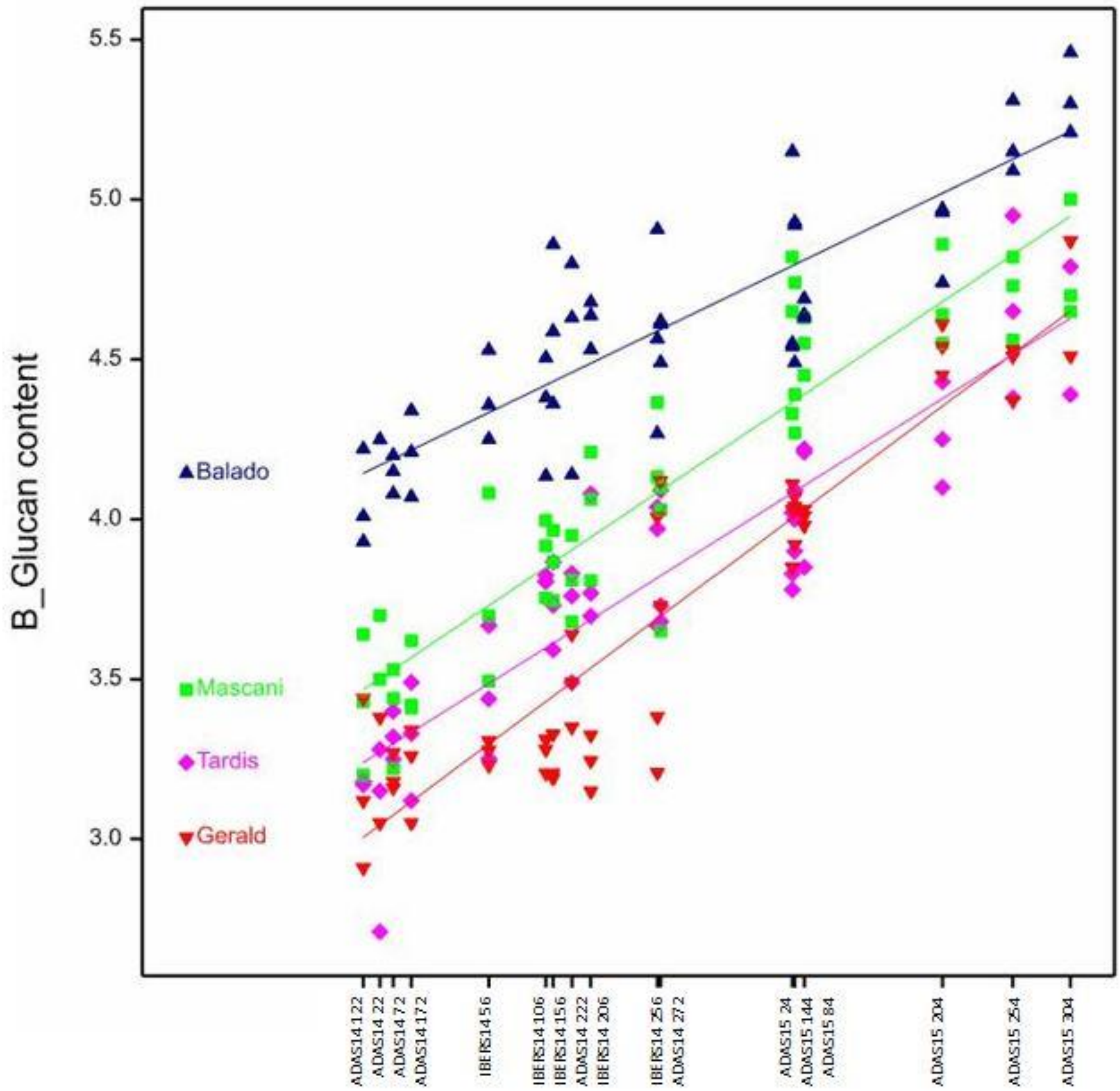


Figure 4.14.b *Joint regression graph for β-glucan content (%) of four winter oat varieties and total nitrogen at ADAS14, IBERS14 and ADAS15. 2013- 2014 and 2014-2015 harvest seasons*.

Joint regression analysis (figure 4.14.b. table 4.12.a) showed statistically significant differences in sensitivity values and total nitrogen levels (p-value<0.001). Balado showed a lower sensitivity value, 0.76, and therefore higher stability against changes in total nitrogen levels, whilst Gerald with a sensitivity value of 1.18, showed lower stability against changes in total nitrogen levels. ADAS15 304 kg/ha total nitrogen showed higher β-glucan content 4.9%, whilst ADAS14 122 kg/ha total nitrogen; showed the lower, 3.5% (table 4.12.a).

Table 4.12.a *Mean β-glucan content (%) ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	β-Glucan content (%)	s.e.m.	Rank
ADAS 2015	304	4.86	0.100	1
ADAS 2015	254	4.75	0.090	2
ADAS 2015	204	4.61	0.077	3
ADAS 2015	84	4.34	0.084	4
ADAS 2015	144	4.32	0.109	5
ADAS 2015	24	4.31	0.127	6
ADAS 2014	272	4.05	0.105	7
IBERS 2014	256	4.05	0.143	8
IBERS 2014	206	3.91	0.153	9
ADAS 2014	222	3.88	0.129	10
IBERS 2014	156	3.84	0.152	11
IBERS 2014	106	3.82	0.119	12
IBERS 2014	56	3.71	0.136	13
ADAS 2014	172	3.56	0.123	14
ADAS 2014	72	3.52	0.394	15
ADAS 2014	22	3.50	0.163	16
ADAS 2014	122	3.46	0.119	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivity	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*

As with protein content, a positive effect was found on β-glucan content (table 4.12.b) with higher fertilizer levels. Balado was the variety least affected e.g. with a 7.6% higher *β*-glucan content, 4.6% at level 5, respect level 0, 4.3% at ADAS 2014 (table 4.12.b). On the other hand, Mascani was the most affected with an increase of the 29.9% higher β-glucan content at level 4 at IBERS 2014 in comparison with level 0 at the same site (table 4.12.b).

Table 4.12.b *Mean β-glucan content (%) ± s.e.m. at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied. % refers to the change between the respective 0 level for each site and variety and the nitrogen level applied.*

		ADAS14			**ADAS15**			**IBERS14**		
	Nitrogen level	***B-Glucan content***	***s.e.m***	***% Increase***	***B-Glucan content***	***s.e.m***	***% Increase***	***B-Glucan content***	***s.e.m***	***% Increase***
Balado	0	4.25	0.000	0.0	4.75	0.202	0.0	4.38	0.018	0.0
	1	4.14	0.035	-2.5	4.65	0.019	-2.0	4.34	0.024	-0.9
	2	4.05	0.086	-4.6	4.78	0.145	0.7	4.60	0.032	5.1
	3	4.21	0.078	-1.0	4.89	0.075	3.0	4.62	0.010	5.4
	4	4.52	0.198	6.4	5.18	0.066	9.2	4.58	0.041	4.6
	5	4.57	0.042	10.4	5.32	0.073	12.1			
Gerald	0	3.22	0.135	0.0	4.00	0.077	0.0	3.27	0.005	0.0
	1	3.20	0.034	-0.4	4.01	0.015	0.3	3.27	0.007	-0.2
	2	3.16	0.154	-1.8	4.01	0.046	0.3	3.24	0.010	-0.9
	3	3.22	0.086	0.1	4.53	0.046	13.4	3.24	0.011	-1.0
	4	3.49	0.084	8.7	4.47	0.050	11.8	3.53	0.054	8.0
	5	3.93	0.159	22.1	4.75	0.120	18.8			
Mascani	0	3.60	0.082	0.0	4.60	0.144	0.0	3.76	0.039	0.0
	1	3.40	0.092	-5.6	4.54	0.052	-1.2	3.89	0.016	3.5
	2	3.42	0.127	-4.9	4.47	0.141	-2.9	3.86	0.014	2.7
	3	3.48	0.068	-3.2	4.68	0.092	1.8	4.03	0.026	7.1
	4	3.81	0.078	5.9	4.70	0.076	2.2	4.25	0.021	13.1
	5	3.93	0.140	9.1	4.78	0.109	4.0			
Tardis	0	3.05	0.172	0.0	3.88	0.073	0.0	3.45	0.027	0.0
	1	3.32	0.043	9.1	4.09	0.122	5.6	3.81	0.001	10.4
	2	3.18	0.003	4.3	4.00	0.055	3.1	3.73	0.018	8.0
	3	3.31	0.107	8.8	4.26	0.095	9.9	3.85	0.026	11.5
	4	3.69	0.104	21.2	4.66	0.165	20.2	3.89	0.025	12.7
	5	3.83	0.129	25.8	4.59	0.163	18.4			
Significance (G)			*Non-significant*			*Non-significant*			*Non-significant*	
Significance (N)			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (GxN)			*Non-significant*			*Non-significant*			*Non-significant*	

4.3.7 Grain and groat size and shape parameters

Mean grain area (mm^2), length (mm) and width (mm) were determined using image analysis (MARVIN, (Sensorik and GmbH, 2001)) for the four winter oat varieties, Balado, Gerald, Mascani and Tardis at each level of nitrogen fertilization (table 4.2.a and 4.2.b) at the three trials described above (ADAS 2014 and 2015, and IBERS 2014). In addition, the ratio between the length and width (grain ratio) was calculated as a measure of grain shape.

Area (mm^2), width (mm) and length (mm) of the grain displayed statistically significant differences (p-value<0.001) between varieties, levels of fertilizer (table 4.13) and interaction between the two factors (table 4.13). Mean grain area and length values from all sites (table 4.13) increased with increasing levels of nitrogen applied. However, mean grain width displayed lower values at higher levels of nitrogen at ADAS 2014 and 2015. This trend was much more marked at IBERS 2014 which displayed a greater effect on grain area, length and width, with higher mean values when increasing levels of total nitrogen. It is particularly interesting that at IBERS 2014 and ADAS 2015, with the same amount of total nitrogen, i.e. summing up the nitrogen present in the soil (SMN) plus the N applied, there were different rate of increase between level 0 and higher levels of nitrogen. The consequence of these increases varying in grain length and grain width at higher levels of nitrogen was that the mean grain ratio decreased significantly with increasing nitrogen fertiliser levels (table 4.13). Grain ratio was also significantly different between varieties.

Table 4.13 *Mean area (mm²), width (mm) and length (mm) from grain of the four winter oat varieties under each level of nitrogen fertilizer (kg/ha) ADAS2014, 2015 and IBERS 2014 harvest season. % refers to the change between the respective 0 level for each site and variety and the nitrogen level applied. *Nitrogen levels applied specified in table 2.2.a.*

ADAS14	***N applied kg/ha***	***N total kg/ha***	***Area Grain mm^2***	***s.e.m.***	***% Increase***	***Width Grain mm***	***s.e.m.***	***% Increase***	***Length Grain mm***	***s.e.m.***	***% Increase***	***Grain ratio***	***s.e.m.***	***% Increase***
0	0	22	27.26	0.185	0.0	3.10	0.005	0.0	12.37	0.094	0.0	0.26	0.002	0.0
1	50	72	27.47	0.187	0.8	3.15	0.008	1.4	12.21	0.077	-1.3	0.26	0.001	0.5
2	100	122	27.18	0.231	-0.3	3.13	0.010	1.0	12.04	0.092	-2.6	0.26	0.002	1.5
3	150	172	27.35	0.234	0.0	3.14	0.009	1.0	12.12	0.093	-2.0	0.26	0.002	0.9
4	200	222	27.99	0.226	2.7	3.14	0.009	1.2	12.41	0.092	0.4	0.25	0.001	-1.3
5	250	272	27.72	0.217	1.7	3.09	0.010	-1.9	12.45	0.084	2.0	0.25	0.001	-3.3
Significance (G)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (N)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (GxN)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
ADAS15	***N applied***	***N Total***	***Area Grain***	***s.e.m.***	***% Increase***	***Width Grain***	***s.e.m.***	***% Increase***	***Length Grain***	***s.e.m.***	***% Increase***	***Grain Ratio***	***s.e.m.***	***% Increase***
0	0	24	26.14	0.228	0.0	3.01	0.011	0.0	11.98	0.098	0.0	0.25	0.002	0.0
1	60	84	26.61	0.227	1.8	3.03	0.009	0.6	12.12	0.098	1.2	0.25	0.002	0.6
2	120	144	26.38	0.246	0.9	3.02	0.012	0.0	12.06	0.094	0.7	0.25	0.001	0.7
3	180	204	26.52	0.225	1.4	2.98	0.016	-1.2	12.18	0.073	1.7	0.24	0.001	-3.0
4	230	254	26.93	0.229	3.0	2.99	0.016	-0.9	12.32	0.074	2.8	0.24	0.001	-3.8
5	280	304	26.67	0.016	2.0	2.97	0.086	-1.5	12.24	0.351	2.2	0.24	0.648	-3.8
Significance (G)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (N)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (GxN)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	

IBERS14	***N applied***	***N total***	***Area Grain***	***s.e.m.***	***%***	***Width Grain***	***s.e.m.***	***%***	***Length Grain***	***s.e.m.***	***%***	***Grain ratio***	***s.e.m.***	***%***
0	0	56	26.44	0.969	0.0	3.19	0.025	0.0	11.44	0.405	0.0	0.28	0.106	0.0
1	50	106	27.81	0.954	5.2	3.24	0.023	1.3	11.92	0.413	4.2	0.27	0.110	2.8
2	100	156	27.62	0.960	4.5	3.20	0.031	0.2	11.96	0.431	4.6	0.27	0.125	4.4
3	150	206	28.04	1.009	6.1	3.19	0.026	0.0	12.20	0.466	6.7	0.26	0.137	7.0
4	200	256	28.63	0.996	8.3	3.21	0.029	0.4	12.39	0.440	8.3	0.26	0.132	8.0
Significance (G)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (N)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (GxN)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	

Joint regression analysis (figure 4.15, table 4.14) on grain area showed statistically significant differences in sensitivity values, genotypes and total nitrogen levels (p-value<0.001). Tardis showed a lower sensitivity value, 0.68, and therefore higher stability against total nitrogen levels whilst Balado with a sensitivity value of 1.51, showed lower stability against changes in total nitrogen levels. IBERS14 256 kg/ha total nitrogen showed higher grain area 28.58 mm^2, whilst ADAS15 24 kg/ha total nitrogen; showed the lower, 26.19 mm^2 (table 4.14).

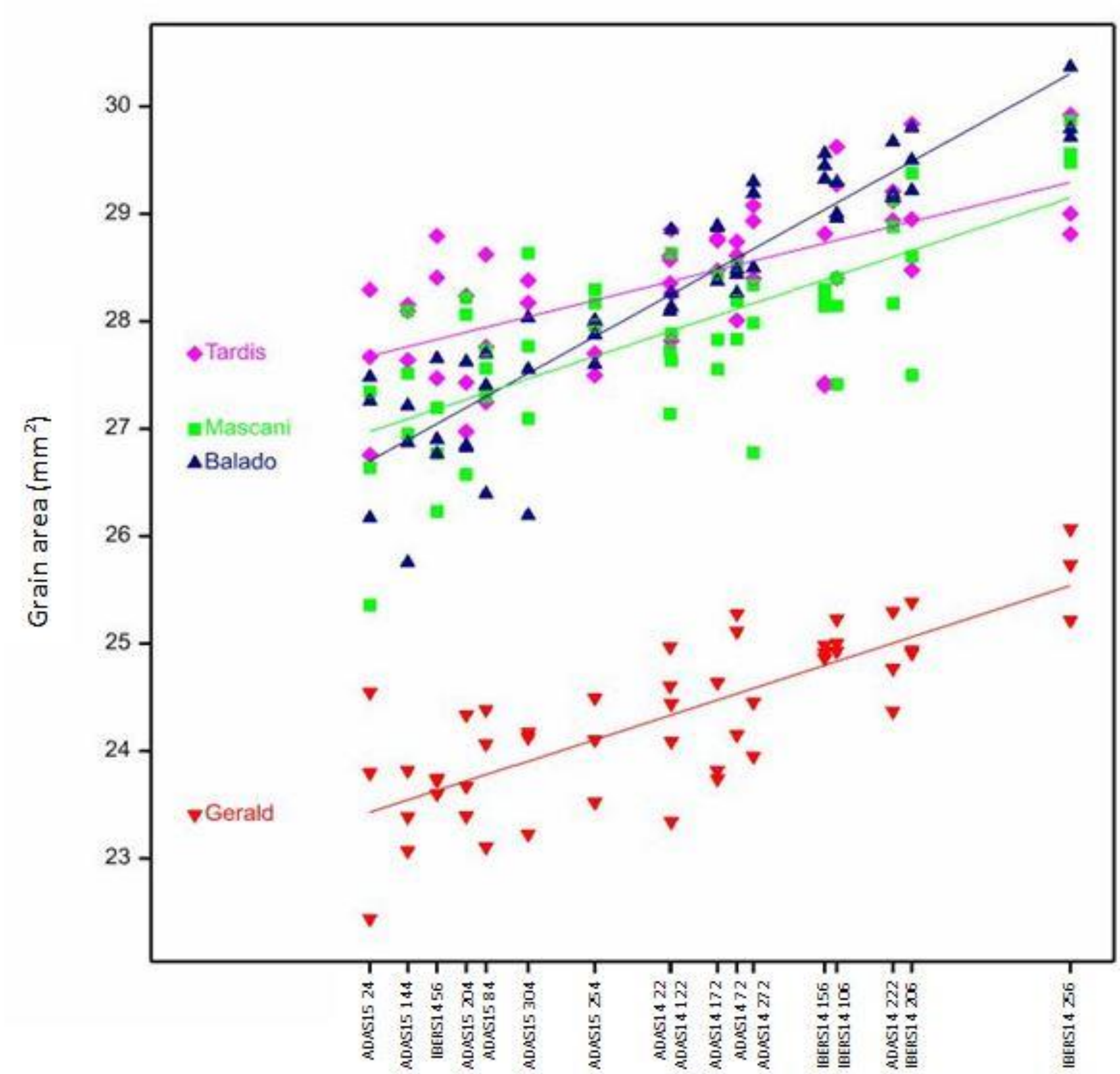


Figure 4.15 *Joint regression graph for grain area (mm^2) of four winter oat varieties and total nitrogen at ADAS14, IBERS14 and ADAS15. 2013- 2014 and 2014-2015 harvest seasons*.

Table 4.14 *Mean grain area (mm²) ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Grain Area (mm^2)	s.e.m.	Rank
IBERS 2014	256	28.58	0.649	1
IBERS 2014	206	28.04	0.672	2
ADAS 2014	222	27.97	1.957	3
IBERS 2014	106	27.78	0.623	4
IBERS 2014	156	27.74	0.627	5
ADAS 2014	272	27.5	0.664	6
ADAS 2014	72	27.44	0.574	7
ADAS 2014	172	27.37	0.586	8
ADAS 2014	122	27.22	0.576	9
ADAS 2014	22	27.21	0.566	10
ADAS 2015	254	26.96	0.626	11
ADAS 2015	304	26.73	0.688	12
ADAS 2015	84	26.59	0.621	13
ADAS 2015	204	26.52	0.615	14
IBERS 2014	56	26.42	0.637	15
ADAS 2015	144	26.32	0.674	16
ADAS 2015	24	26.19	0.626	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivity	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*

When the effect of nitrogen on the individual varieties is examined, the greatest response of grain area to nitrogen was found for Mascani at IBERS 2014 (figure 4.16), 10.8% at level 4 more area than at level 0. At all sites, Mascani grain area increased up to level 4, whereas the grain size of Tardis and Gerald did not change greatly in response to nitrogen, particularly at ADAS 2014 and ADAS 2015

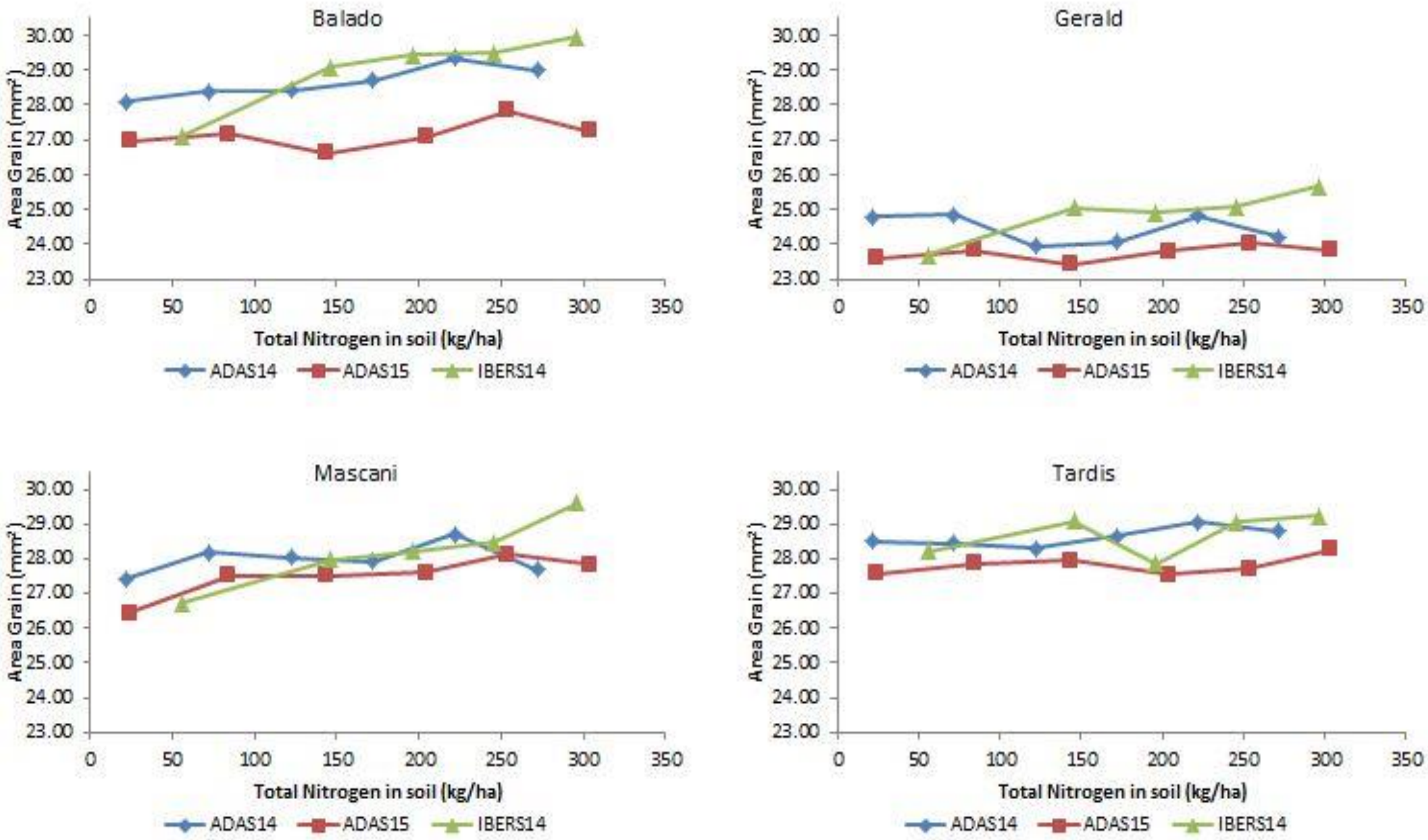


***Figure 4.16** Mean area (mm^2) ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at ADAS 2014 and 2015 and IBERS 2014, harvest seasons.*

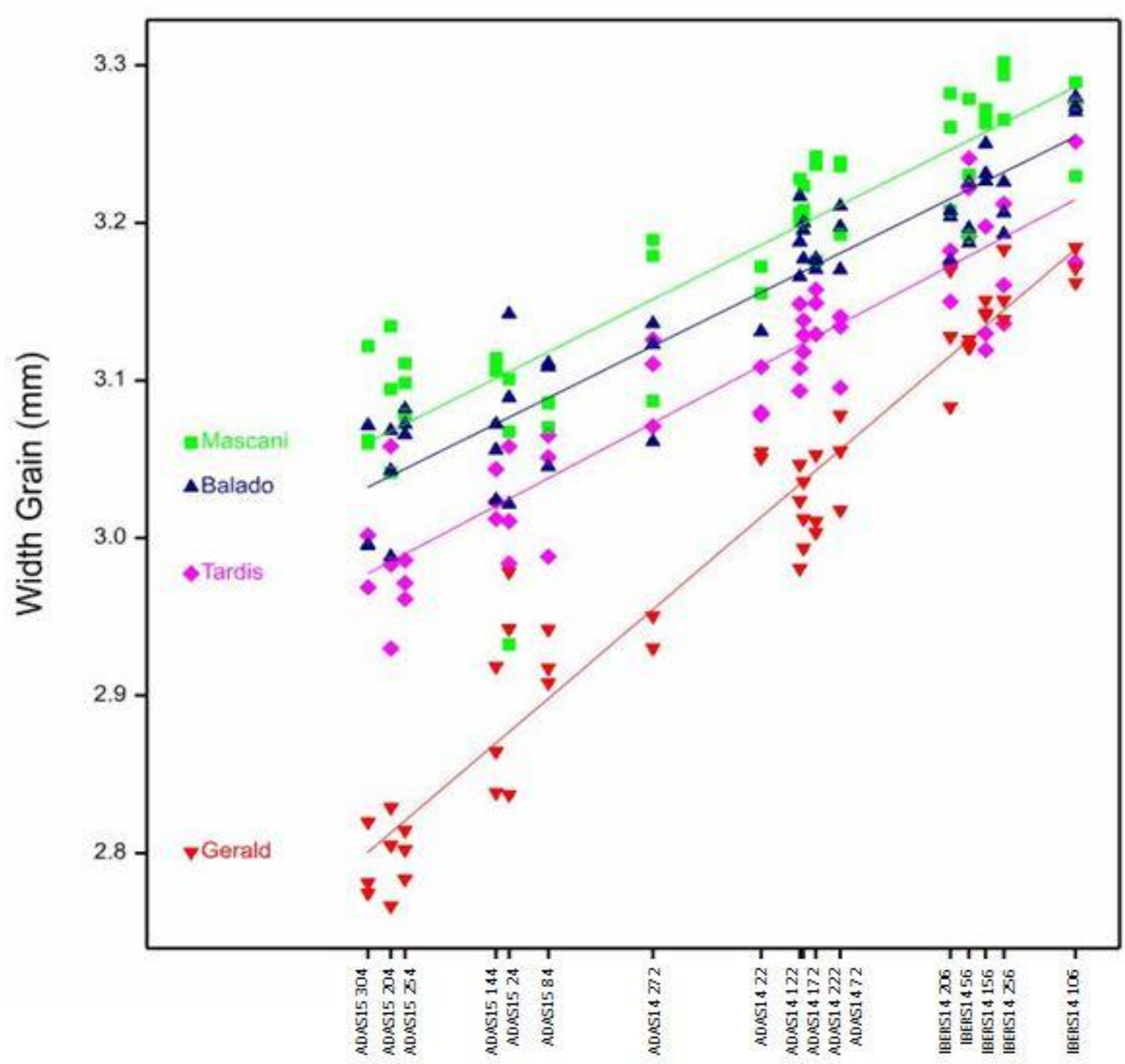


Figure 4.17 *Joint regression graph for grain width (mm) of four winter oat varieties and total nitrogen at ADAS14, IBERS14 and ADAS15. 2013- 2014 and 2014-2015 harvest seasons*

Joint regression analysis (figure 4.17, table 4.15) on grain width showed statistically significant differences in sensitivity values, genotypes and total nitrogen levels (p-value<0.001). Balado showed a lower sensitivity value, 0.83, and therefore higher stability against total nitrogen levels whilst Gerald with a sensitivity value of 1.49, showed lower stability against changes in total nitrogen levels. IBERS14 106 kg/ha total nitrogen showed higher grain width 3.24 mm, whilst ADAS15 304 kg/ha total nitrogen; showed the lower, 2.97 mm (table 4.15).

Table 4.15 *Mean grain width (mm) ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Grain Width (mm)	s.e.m.	Rank
IBERS 2014	106	3.24	0.017	1
IBERS 2014	256	3.21	0.020	2
IBERS 2014	156	3.20	0.021	3
IBERS 2014	56	3.20	0.018	4
IBERS 2014	206	3.19	0.019	5
ADAS 2014	72	3.15	0.026	6
ADAS 2014	222	3.14	0.028	7
ADAS 2014	172	3.13	0.023	8
ADAS 2014	122	3.13	0.024	9
ADAS 2014	22	3.12	0.016	10
ADAS 2014	272	3.08	0.029	11
ADAS 2015	84	3.04	0.026	12
ADAS 2015	24	3.02	0.030	13
ADAS 2015	144	3.02	0.033	14
ADAS 2015	254	2.98	0.043	15
ADAS 2015	204	2.98	0.043	16
ADAS 2015	304	2.97	0.043	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*

Mean grain width (mm) (figure 4.18) displayed a significant nitrogen by variety interaction, with Balado, Gerald and Tardis displaying lower values in general with higher levels of nitrogen fertilizer applied. A slight increase in mean grain width was found at lower levels of nitrogen at certain sites, i.e. level 3 at ADAS 2014, level 1 at ADAS 2015 and at level 2 at IBERS 2014. Mascani however, increased mean grain width in response to nitrogen with a decrease only found at level 5 at ADAS 2014 and ADAS 2015.

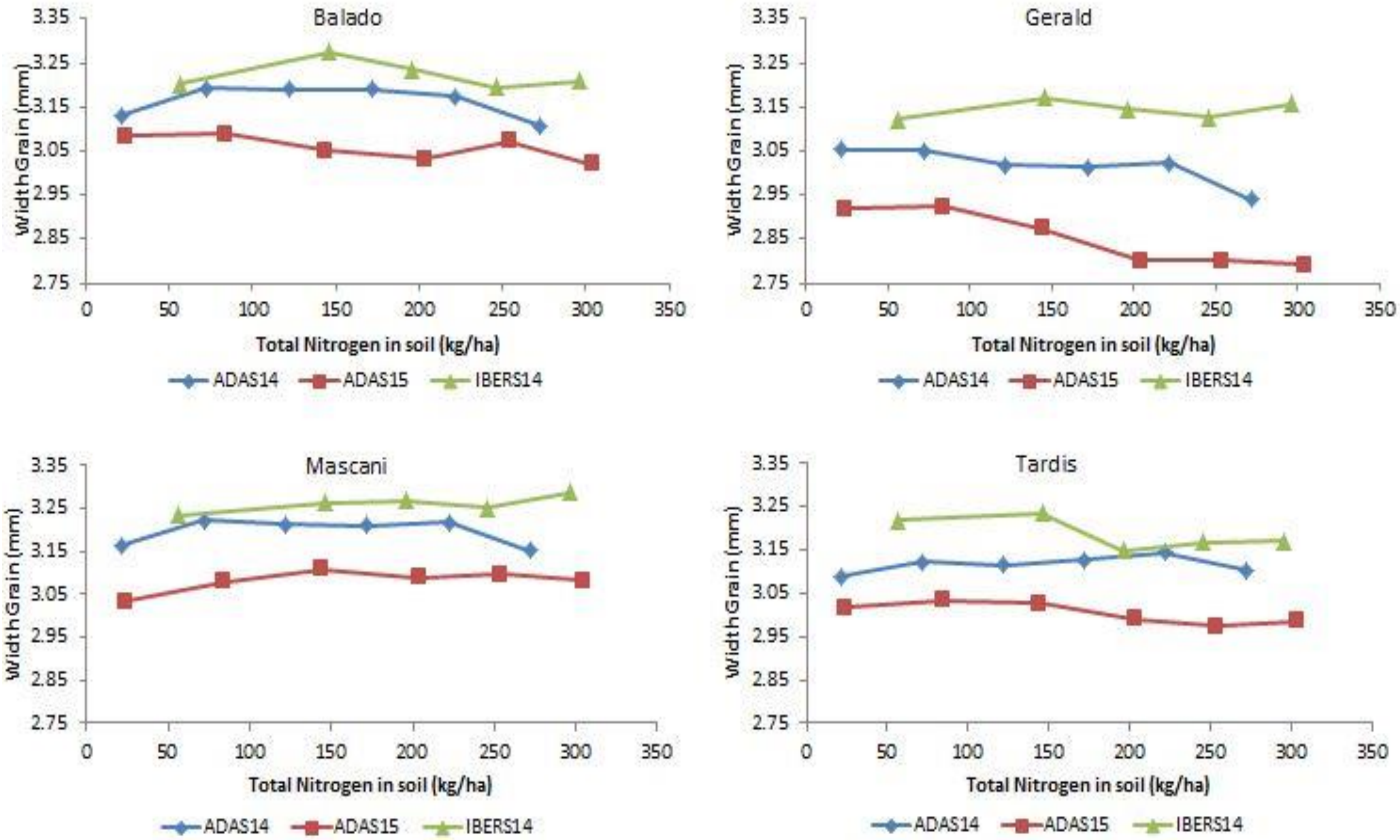


Figure 4.18 *Mean grain width (mm) ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at (ADAS) 2014, 2015 and IBERS 2015, harvest season.*

Joint regression analysis (figure 4.19, table 4.16) on grain length showed no statistically significant differences in sensitivity values, but there were between total nitrogen levels (p-value<0.001). ADAS14 222 kg/ha total nitrogen showed higher grain length 12.42 mm, whilst IBERS14 56 kg/ha total nitrogen; showed the lower, 11.44 mm (table 4.16).

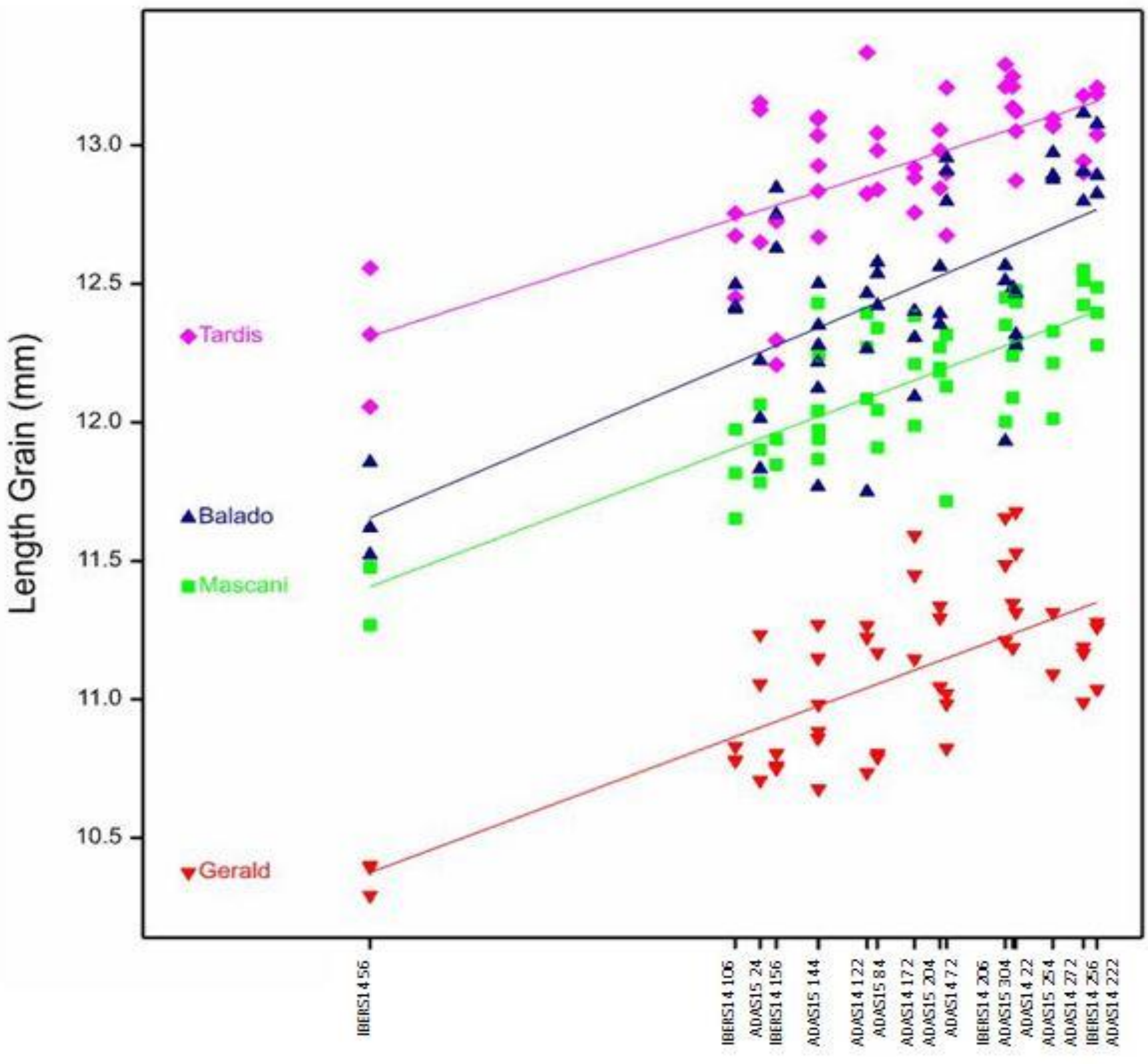


***Figure 4.19** Joint regression graph for grain length (mm) of four winter oat varieties and total nitrogen at ADAS14, IBERS14 and ADAS15. 2013- 2014 and 2014-2015 harvest seasons*

Table 4.16 *Mean grain length (mm) ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Grain Length (mm)	s.e.m.	Rank
ADAS 2014	222	12.42	0.282	1
IBERS 2014	256	12.40	0.284	2
ADAS 2014	272	12.36	0.256	3
ADAS 2015	254	12.31	0.202	4
ADAS 2014	22	12.31	0.287	5
ADAS 2015	304	12.30	0.234	6
IBERS 2014	206	12.22	0.305	7
ADAS 2014	72	12.21	0.235	8
ADAS 2015	204	12.17	0.199	9
ADAS 2014	172	12.12	0.232	10
ADAS 2015	84	12.11	0.267	11
ADAS 2014	122	12.04	0.230	12
ADAS 2015	144	12.04	0.257	13
IBERS 2014	156	11.99	0.279	14
ADAS 2015	24	11.96	0.268	15
IBERS 2014	106	11.93	0.266	16
IBERS 2014	56	11.44	0.264	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*No significant*	*No significant*		*No significant*

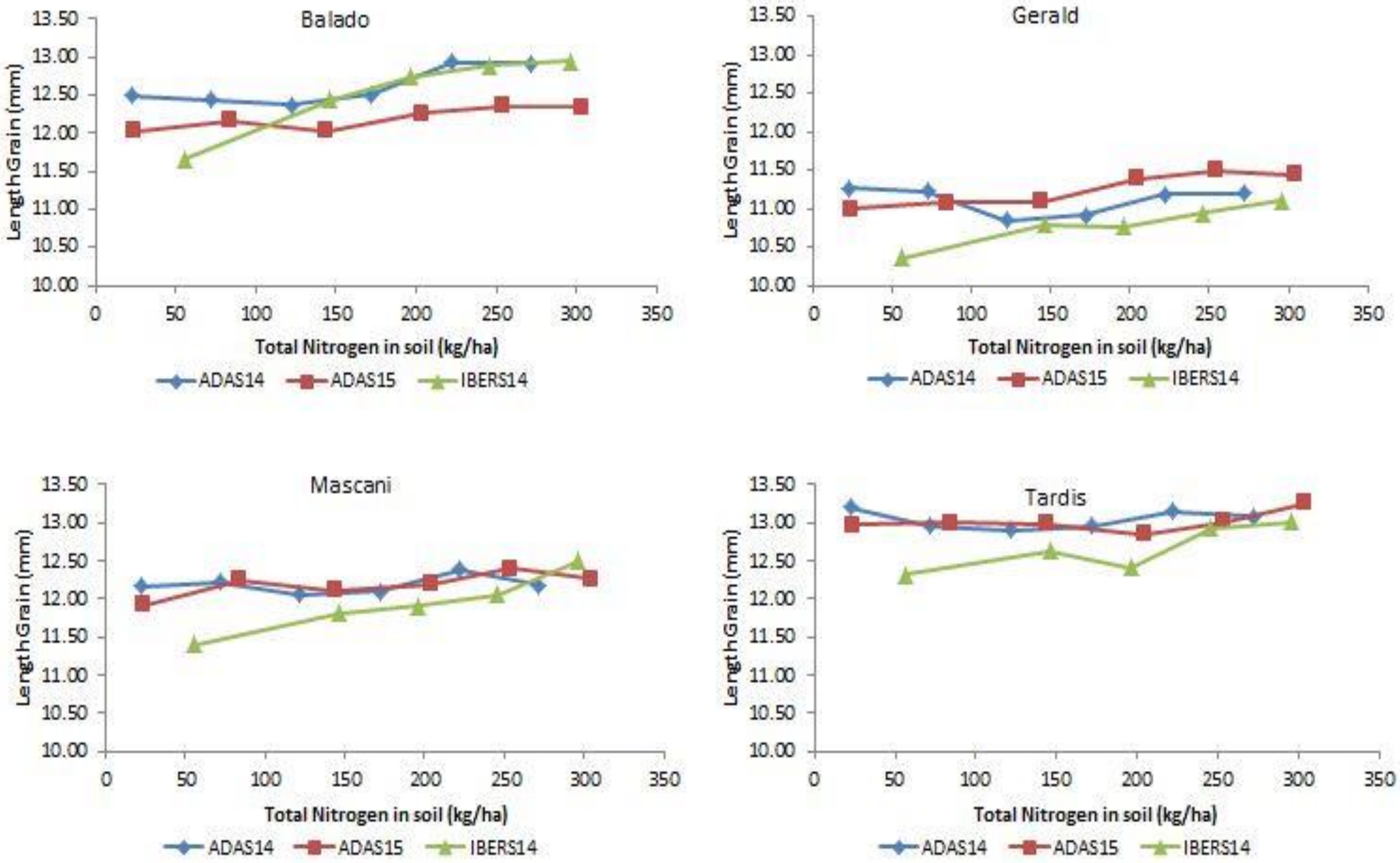


Figure 4.20 *Mean grain length (mm) ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at Rosemaund (ADAS) 2014, 2015 and IBERS 2015, harvest season*

Mean grain length values were overall higher with increasing levels of nitrogen (figure 4.20) for all varieties but Gerald at ADAS 2014. Mean grain length displayed the biggest response to nitrogen at IBERS 2014, where Balado displayed the highest effect in comparison with the rest of varieties, having a 10.9% higher length at level 4 as compared to the level 0 value.

Joint regression analysis (figure 4.21, table 4.17) on grain ratio showed statistically significant differences in sensitivity values and total nitrogen levels (p-value<0.001). IBERS14 56 kg/ha total nitrogen showed higher grain ratio 0.28, whilst ADAS15 304 kg/ha total nitrogen; showed the lower, 0.24 (table 4.17).

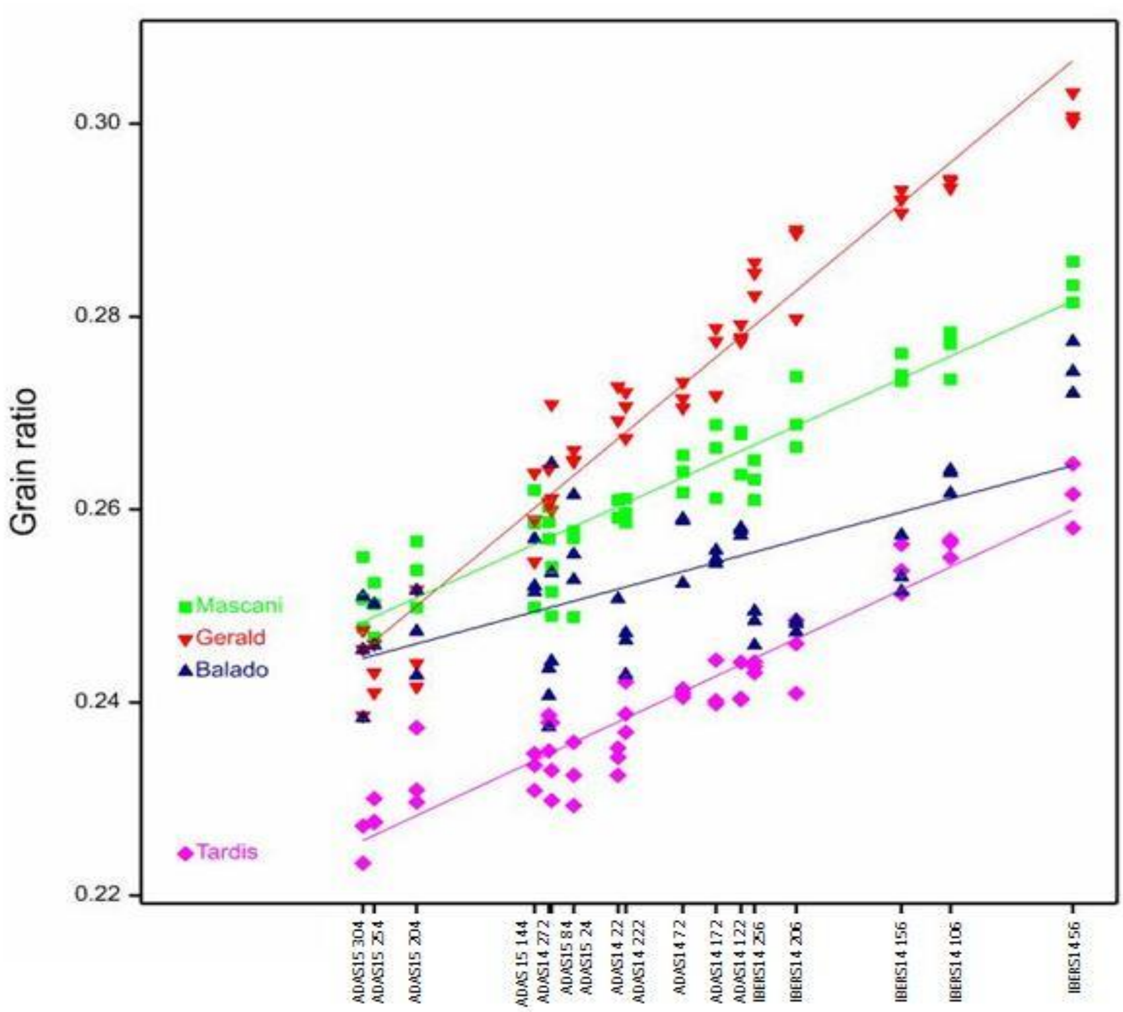


Figure 4.21 *Mean grain ratio ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at Rosemaund (ADAS) 2014, 2015 and IBERS 2015, harvest season.*

Balado showed a lower sensitivity value, 0.53, and therefore higher stability against total nitrogen levels whilst Gerald with a sensitivity value of 1.63, showed lower stability against changes in total nitrogen levels.

Table 4.17 *Mean grain ratio ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Grain Ratio	s.e.m.	Rank
IBERS 2014	56	0.28	0.005	1
IBERS 2014	106	0.27	0.005	2
IBERS 2014	156	0.27	0.006	3
IBERS 2014	206	0.26	0.006	4
IBERS 2014	256	0.26	0.006	5
ADAS 2014	122	0.26	0.002	6
ADAS 2014	172	0.26	0.003	7
ADAS 2014	72	0.26	0.003	8
ADAS 2014	222	0.25	0.003	9
ADAS 2014	22	0.25	0.004	10
ADAS 2015	24	0.25	0.005	11
ADAS 2015	84	0.25	0.004	12
ADAS 2014	272	0.25	0.005	13
ADAS 2015	144	0.25	0.004	14
ADAS 2015	204	0.24	0.003	15
ADAS 2015	254	0.24	0.003	16
ADAS 2015	304	0.24	0.004	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*

Individual groat weight and size parameters (table 4.18) were determined after the same sample of grain analysed above had been dehulled for kernel content and hullability determination. Interestingly, for all parameters measured (thousand groat weight, groat area, groat width, groat length and groat ratio mean values), significant differences were found between varieties and for the interaction of variety and nitrogen (p-value<0.001) but the effect of nitrogen levels was not significant at all sites (p-value>0.05).

Table 4.18 *Mean thousand groat weight, groat area (mm²), width (mm), length (mm) and ratio from groat of the four oat winter varieties under each level of nitrogen fertilizer (kg/ha) Rosemaund 2014 and 2015 and IBERS 2014, harvest season. % refers to the change between the respective 0 level for each site and variety and the nitrogen level applied. *Nitrogen levels applied specified in table 2.2.a.*

ADAS14	***N applied***	***N total***	***TGW Groat***	***% Increase***	s.e.m	***Area Groat***	***% Increase***	s.e.m	***Width Groat***	***% Increase***	s.e.m	***Length Groat***	***% Increase***	s.e.m	***Groat ratio***	***% Increase***	s.e.m
0	0	22	29.30	0.0	0.320	14.92	0.0	0.109	2.64	0.0	0.007	6.85	0.0	0.036	0.39	0.0	0.002
1	50	72	30.93	5.7	0.378	15.46	3.6	0.139	2.66	0.4	0.008	6.98	2.0	0.043	0.38	-1.5	0.002
2	100	122	30.85	5.4	0.446	15.39	3.2	0.162	2.64	-0.3	0.009	6.98	2.0	0.052	0.38	-2.1	0.002
3	150	172	31.30	6.9	0.449	15.72	5.3	0.154	2.65	0.1	0.008	7.11	3.8	0.052	0.37	-3.3	0.002
4	200	222	31.72	8.3	0.421	15.97	7.0	0.143	2.64	-0.1	0.006	7.18	4.8	0.047	0.37	-4.5	0.002
5	250	272	30.97	5.8	0.427	15.82	6.0	0.147	2.59	-2.2	0.009	7.23	5.5	0.048	0.0.36	-7.3	0.002
Significance (G)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (N)				*Non-significant*			*Non-significant*			*Non-significant*			*Non-significant*			*Non-significant*	
Significance (GxN)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
ADAS15	***N applied***	***N total***	***TGW Groat***	***%% Increase***	s.e.m	***Area Groat***	***% Increase***	s.e.m	***Width Groat***	***% Increase***	s.e.m	***Length Groat***	***%% Increase***	s.e.m	***Groat ratio***	***%% Increase***	s.e.m
0	0	24	29.66	0.0	0.365	14.52	0.0	0.132	2.53	0.0	0.008	6.89	0.0	0.045	0.37	0.0	0.072
1	60	84	30.72	3.6	0.404	14.81	2.0	0.154	2.53	0.3	0.009	6.98	1.1	0.050	0.36	-0.8	0.072
2	120	144	30.72	3.6	0.493	14.94	2.9	0.158	2.51	-0.6	0.010	7.04	2.3	0.046	0.36	-2.8	0.066
3	180	204	29.73	0.2	0.594	14.96	3.0	0.180	2.47	-2.4	0.013	7.15	3.8	0.047	0.34	-6.0	0.061
4	230	254	30.19	1.8	0.678	15.16	4.4	0.192	2.48	-2.1	0.014	7.19	4.4	0.052	0.34	-6.2	0.170
5	280	304	28.84	-2.8	0.200	14.93	2.8	0.013	2.45	-3.2	0.059	7.15	3.8	0.044	0.34	-6.7	0.033
Significance (G)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (N)				*Non-significant*			*Non-significant*			*Non-significant*			*Non-significant*			*Non-significant*	
Significance (GxN)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	

IBERS14	***N applied***	***N total***	***TGW Groat***	***% Increase***	s.e.m	***Area Groat***	***% Increase***	s.e.m	***Width Groat***	***% Increase***	s.e.m	***Length Groat***	***% Increase***		***Groat ratio***	***% Increase***	s.e.m
0	0	56	33.48	0.0	0.167	16.25	0.0	0.624	2.74	0.0	0.031	7.13	0.0	0.210	0.38	0.0	0.076
1	50	106	33.29	-0.6	0.413	16.23	-0.1	0.300	2.74	0.2	0.031	7.09	0.5	0.128	0.39	0.7	0.053
2	100	156	33.65	0.5	2.561	16.41	1.0	0.960	2.74	0.3	0.042	7.11	0.0	0.305	0.38	0.3	0.096
3	150	206	32.00	-4.4	1.058	15.86	-2.5	0.296	2.71	-0.9	0.015	7.04	1.3	0.134	0.38	0.4	0.047
4	200	256	34.08	1.8	1.887	16.64	2.4	0.681	2.74	0.0	0.028	7.27	-1.9	0.222	0.37	-1.9	0.071
Significance (G)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	
Significance (N)				*Non-significant*			*Non-significant*			*Non-significant*			*Non-significant*			*Non-significant*	
Significance (GxN)				*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*			*p-value<0.001*	

Joint regression analysis (figure 4.22, table 4.19) on thousand groat weight showed statistically significant differences in sensitivity values and total nitrogen levels (p-value<0.001). IBERS14 106 kg/ha total nitrogen showed higher thousand groat weight 34.58 g, whilst ADAS15 304 kg/ha total nitrogen; showed the lower, 28.86 g (table 4.18). Mascani showed a lower sensitivity value, 0.28, and therefore higher stability against total nitrogen levels whilst Gerald with a sensitivity value of 2.11, showed lower stability against changes in total nitrogen levels.

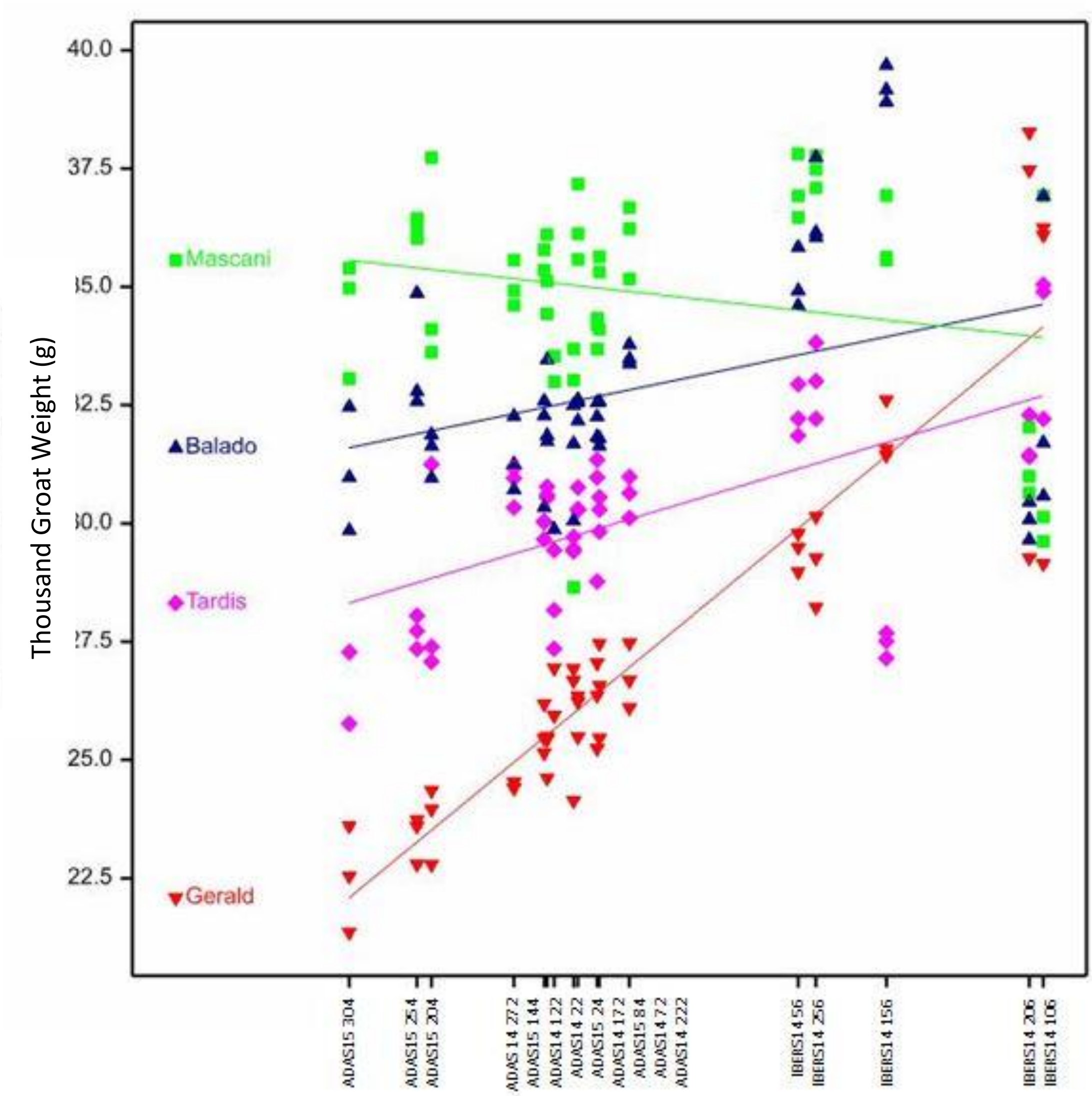


Figure 4.22 *Mean thousand groat weight ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at Rosemaund (ADAS) 2014, 2015 and IBERS 2015, harvest season.*

Table 4.19 *Mean thousand groat weight (g) ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Thousand Groat Weight (g)	s.e.m.	Rank
IBERS 2014	106	34.58	1.067	1
IBERS 2014	206	34.46	1.021	2
IBERS 2014	156	33.29	1.647	3
IBERS 2014	256	32.71	1.231	4
IBERS 2014	56	32.56	1.081	5
ADAS 2014	222	31.17	1.288	6
ADAS 2014	72	30.92	1.158	7
ADAS 2015	84	30.90	1.106	8
ADAS 2014	172	30.75	1.123	9
ADAS 2015	24	30.71	0.999	10
ADAS 2014	22	30.55	2.775	11
ADAS 2014	122	30.49	1.116	12
ADAS 2015	144	30.47	1.349	13
ADAS 2014	272	30.22	1.308	14
ADAS 2015	204	29.54	1.628	15
ADAS 2015	254	29.42	1.856	16
ADAS 2015	304	28.86	1.775	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*

Joint regression analysis (figure 4.23, table 4.20) on groat area showed statistically significant differences in sensitivity values and total nitrogen levels (p-value<0.001). IBERS14 156 kg/ha total nitrogen showed higher groat area 16.99 mm^2, whilst ADAS15 24 kg/ha total nitrogen; showed the lower, 14.78 mm^2 (table 4.20). Tardis showed a lower sensitivity value, 0.13, and therefore higher stability against total nitrogen levels whilst Gerald with a sensitivity value of 1.91, showed lower stability against changes in total nitrogen levels.

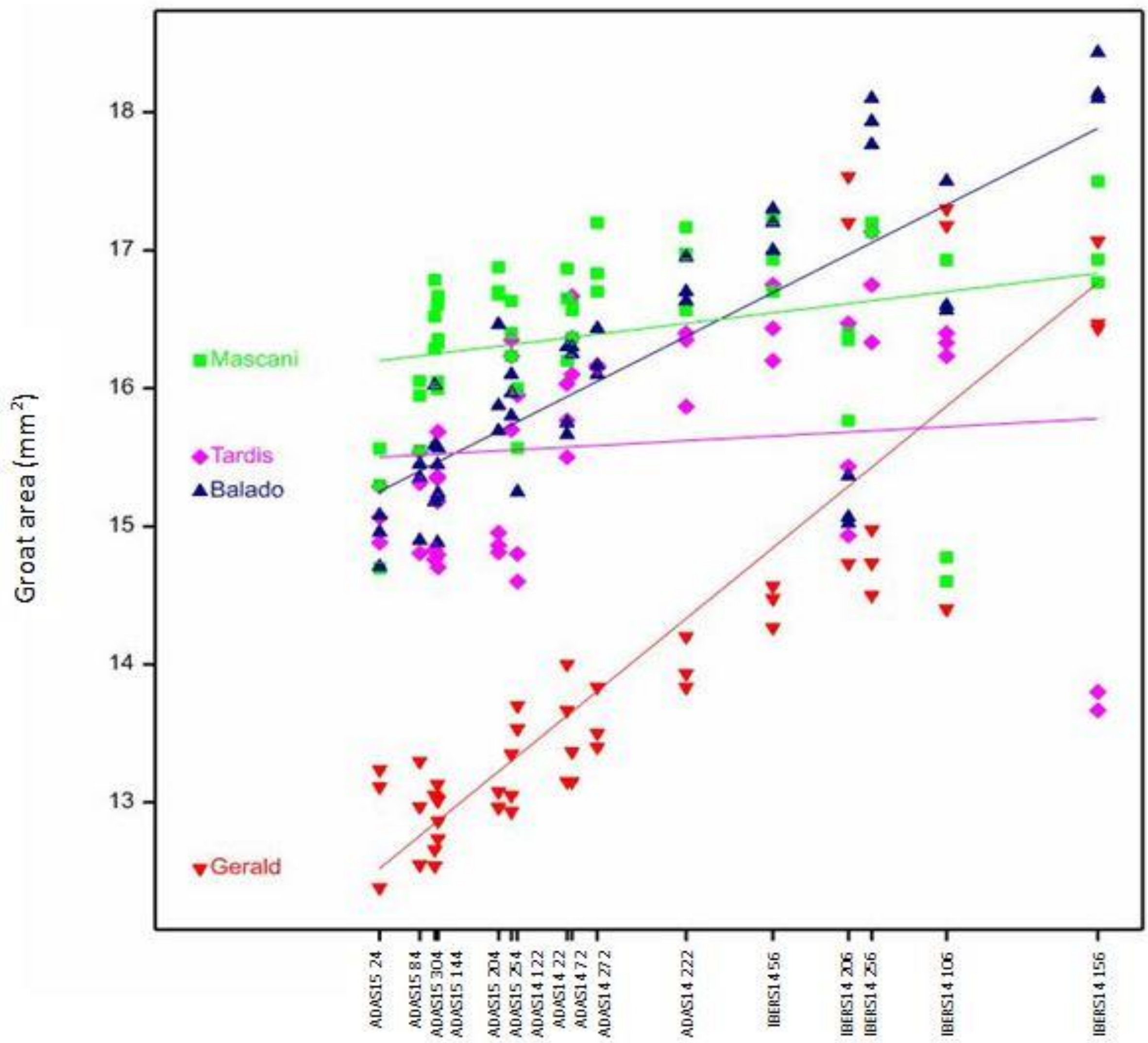


Figure 4.23 *Mean groat area (mm^2) ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at Rosemaund (ADAS) 2014, 2015 and IBERS 2015, harvest season.*

Table 4.20 *Mean groat area (mm²) ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Groat Area (mm^2)	s.e.m.	Rank
IBERS 2014	156	16.99	0.620	1
IBERS 2014	106	16.53	0.378	2
IBERS 2014	256	16.30	0.442	3
IBERS 2014	206	16.22	0.327	4
IBERS 2014	56	15.99	0.405	5
ADAS 2014	222	15.72	0.438	6
ADAS 2014	172	15.45	0.386	7
ADAS 2014	272	15.37	0.451	8
ADAS 2014	72	15.36	0.425	9
ADAS 2014	22	15.20	0.335	10
ADAS 2014	122	15.18	0.405	11
ADAS 2015	254	15.14	0.526	12
ADAS 2015	204	14.96	0.492	13
ADAS 2015	144	14.96	0.433	14
ADAS 2015	304	14.95	0.547	15
ADAS 2015	84	14.90	0.422	16
ADAS 2015	24	14.78	0.361	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*

The varieties showed variable results for mean groat area (figure 4.24). Balado and Mascani mean groat area (figure 4.24) increased with higher levels of nitrogen, although at intermediate levels, 4 at ADAS 2014 and 2015, and level 3 at IBERS 2014, they had the highest mean groat areas. The mean groat area of Gerald was relatively stable across nitrogen treatments; only at IBERS 2014 was there an increase with higher levels of nitrogen applied, with a decrease at the highest level. At ADAS, in both years, the overall result was a lower mean groat area at level 5 with respect to results obtained at level 0. The mean groat area of Tardis increased in response to nitrogen at ADAS 2014, reaching 8.3% higher value when compared to level 0. However, the opposite effect was found at ADAS 2015 and IBERS 2014 (figure 4.24).

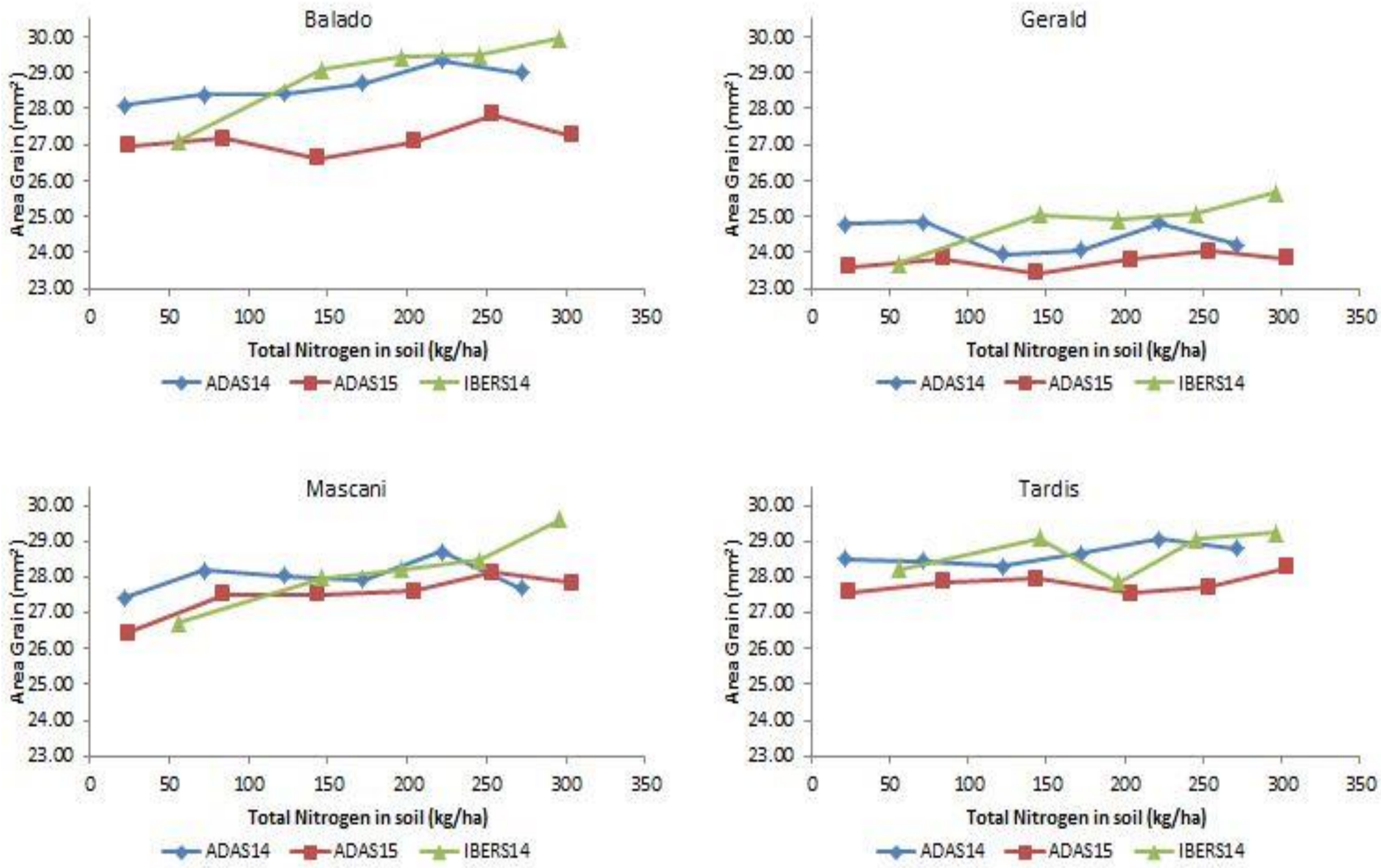


Figure 4.24 *Mean groat area (mm^2) ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at ADAS 2014, 2015 and IBERS 2014, harvest season*

Joint regression analysis (figure 4.25, table 4.21) on groat width showed statistically significant differences in sensitivity values and total nitrogen levels (p-value<0.001). IBERS14 106 kg/ha total nitrogen showed higher groat width 2.75 mm, whilst ADAS15 304 kg/ha total nitrogen; showed the lower, 2.45 mm (table 4.21). Tardis showed a lower sensitivity value, 0.72, and therefore higher stability against total nitrogen levels whilst Gerald with a sensitivity value of 1.29, showed lower stability against changes in total nitrogen levels.

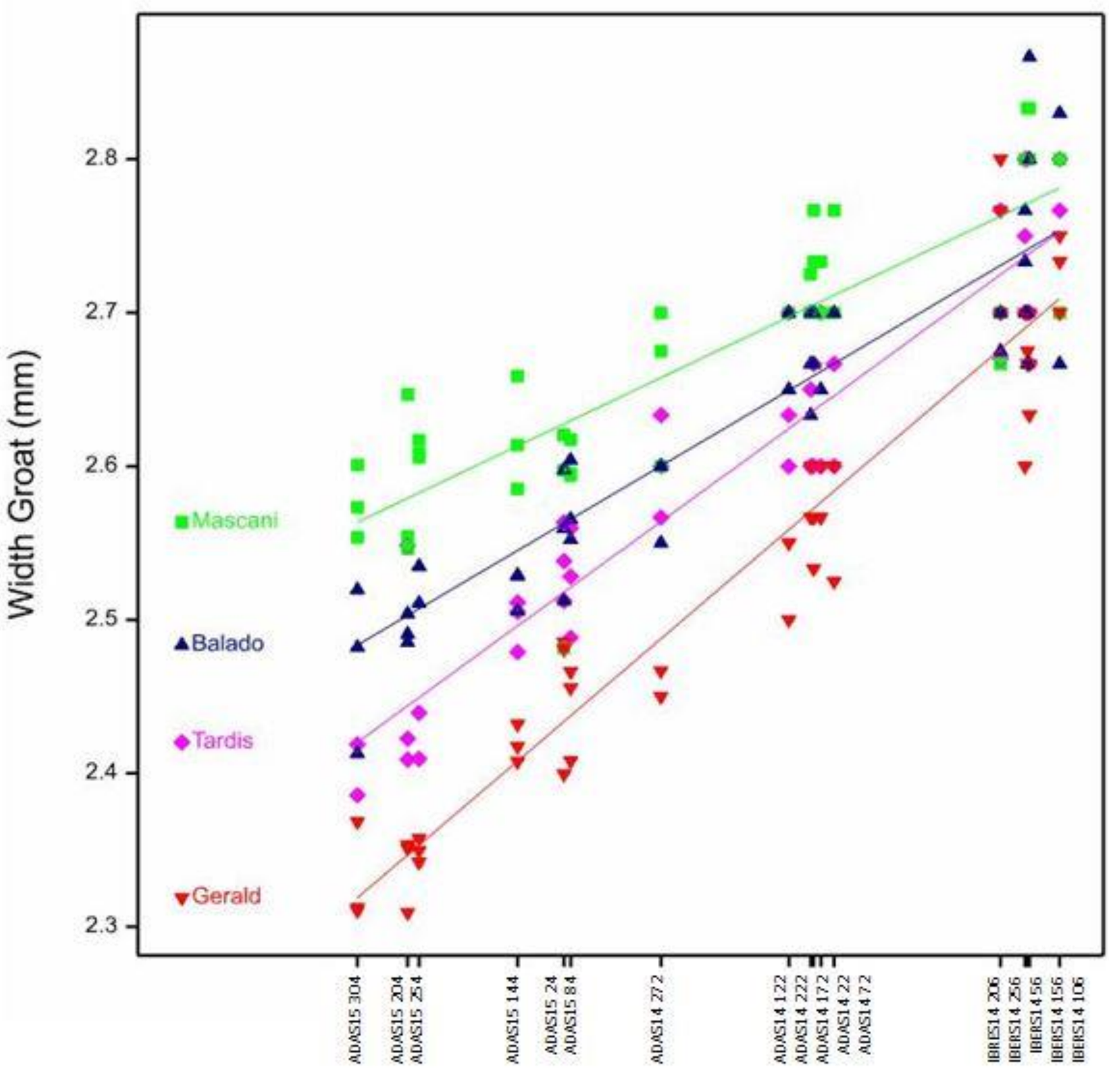


Figure 4.25 *Mean groat width (mm) ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at Rosemaund (ADAS) 2014, 2015 and IBERS 2015, harvest season.*

Table 4.21 *Mean groat width (mm) ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Groat Width (mm)	s.e.m.	Rank
IBERS 2014	106	2.75	0.020	1
IBERS 2014	156	2.74	0.028	2
IBERS 2014	56	2.74	0.022	3
IBERS 2014	256	2.73	0.022	4
IBERS 2014	206	2.72	0.015	5
ADAS 2014	72	2.65	0.024	6
ADAS 2014	22	2.65	0.022	7
ADAS 2014	172	2.64	0.021	8
ADAS 2014	222	2.64	0.020	9
ADAS 2014	122	2.63	0.023	10
ADAS 2014	272	2.58	0.027	11
ADAS 2015	84	2.54	0.024	12
ADAS 2015	24	2.54	0.022	13
ADAS 2015	144	2.52	0.027	14
ADAS 2015	254	2.47	0.037	15
ADAS 2015	204	2.47	0.036	16
ADAS 2015	304	2.45	0.036	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*

A significant effect of variety was found for mean groat width with Balado and Mascani having the widest groats. A significant interaction between variety and nitrogen was found. All varieties at all sites displayed lower mean groat width with increasing levels of nitrogen, except for Balado at IBERS 2014 (table 4.22). This diminishing effect was particularly strong at level 5 ADAS 2014 for Gerald and at level 5 ADAS 2015 for Tardis, each compared with their respective level 0 mean groat width values.

***Table 4.22** Mean groat width (mm), of the four oat winter varieties under each level of nitrogen fertilizer (kg/ha) ADAS 2014 and 2015 and IBERS 2014 harvest season. % refers to the change between the respective 0 level for each site and variety and the nitrogen level applied. *Nitrogen levels applied specified in table 2.2.a.and b.*

Variety	*N level*	*ADAS14*			*ADAS15*			*IBERS14*		
		Width Groat	*%*	*s.e.m.*	*Width Groat*	*%*	*s.e.m.*	*Width Groat*	*%*	*s.e.m.*
Balado	0	2.65	0.0	0.000	2.56	0.0	0.006	2.68	0.0	0.002
	1	2.70	1.9	0.000	2.57	0.7	0.003	2.72	1.2	0.012
	2	2.68	1.3	0.017	2.52	-1.4	0.002	2.82	5.0	0.005
	3	2.68	1.0	0.011	2.49	-2.5	0.001	2.69	0.1	0.002
	4	2.67	0.6	0.019	2.53	-1.2	0.002	2.73	1.7	0.004
	5	2.58	-2.5	0.017	2.47	-3.3	0.007			
Gerald	0	2.58	0.0	0.014	2.46	0.0	0.006	2.68	0.0	0.002
	1	2.58	-0.3	0.025	2.44	-0.5	0.004	2.73	1.8	0.003
	2	2.52	-2.6	0.017	2.42	-1.5	0.002	2.68	-0.5	0.004
	3	2.56	-1.1	0.011	2.34	4.8	0.003	2.76	2.8	0.007
	4	2.58	-0.2	0.011	2.35	-4.3	0.001	2.68	-0.5	0.007
	5	2.46	-4.8	0.007	2.33	-5.1	0.004			
Mascani	0	2.72	0.0	0.014	2.57	0.0	0.010	2.81	0.0	0.002
	1	2.72	0.2	0.022	2.60	1.4	0.002	2.73	-2.8	0.007
	2	2.70	-0.6	0.000	2.62	2.1	0.005	2.81	0.0	0.002
	3	2.73	0.6	0.019	2.58	0.6	0.007	2.69	-4.3	0.002
	4	2.71	-0.3	0.008	2.61	1.7	0.001	2.80	-0.4	0.000
	5	2.66	-2.1	0.030	2.58	0.4	0.003			
Tardis	0	2.63	0.0	0.033	2.54	0.0	0.003	2.77	0.0	0.007
	1	2.62	-0.4	0.022	2.53	-0.5	0.005	2.79	0.8	0.002
	2	2.64	0.4	0.029	2.50	-1.6	0.002	2.68	-3.2	0.002
	3	2.62	-0.4	0.022	2.46	-3.1	0.010	2.71	-2.0	0.006
	4	2.62	-0.6	0.017	2.42	-4.7	0.002	2.75	-0.6	0.006
	5	2.60	-1.3	0.019	2.40	-5.4	0.003			
Significance (G)		*p-value<0.001*			*p-value<0.001*			*p-value<0.001*		
Significance (N)		*Non-significant*			*Non-significant*			*Non-significant*		
Significance (GxN)		*p-value<0.001*			*p-value<0.001*			*p-value<0.001*		

Joint regression analysis (figure 4.26, table 4.23) on groat length showed statistically significant differences in sensitivity values and total nitrogen levels (p-value<0.001). IBERS14 156 kg/ha total nitrogen showed higher groat length 7.49 mm, whilst ADAS15 122 kg/ha total nitrogen; showed the lower, 6.96 mm (table 4.23). Tardis showed a lower sensitivity value, 0.23, and therefore higher stability against total nitrogen levels whilst Gerald with a sensitivity value of 2.30, showed lower stability against changes in total nitrogen levels.

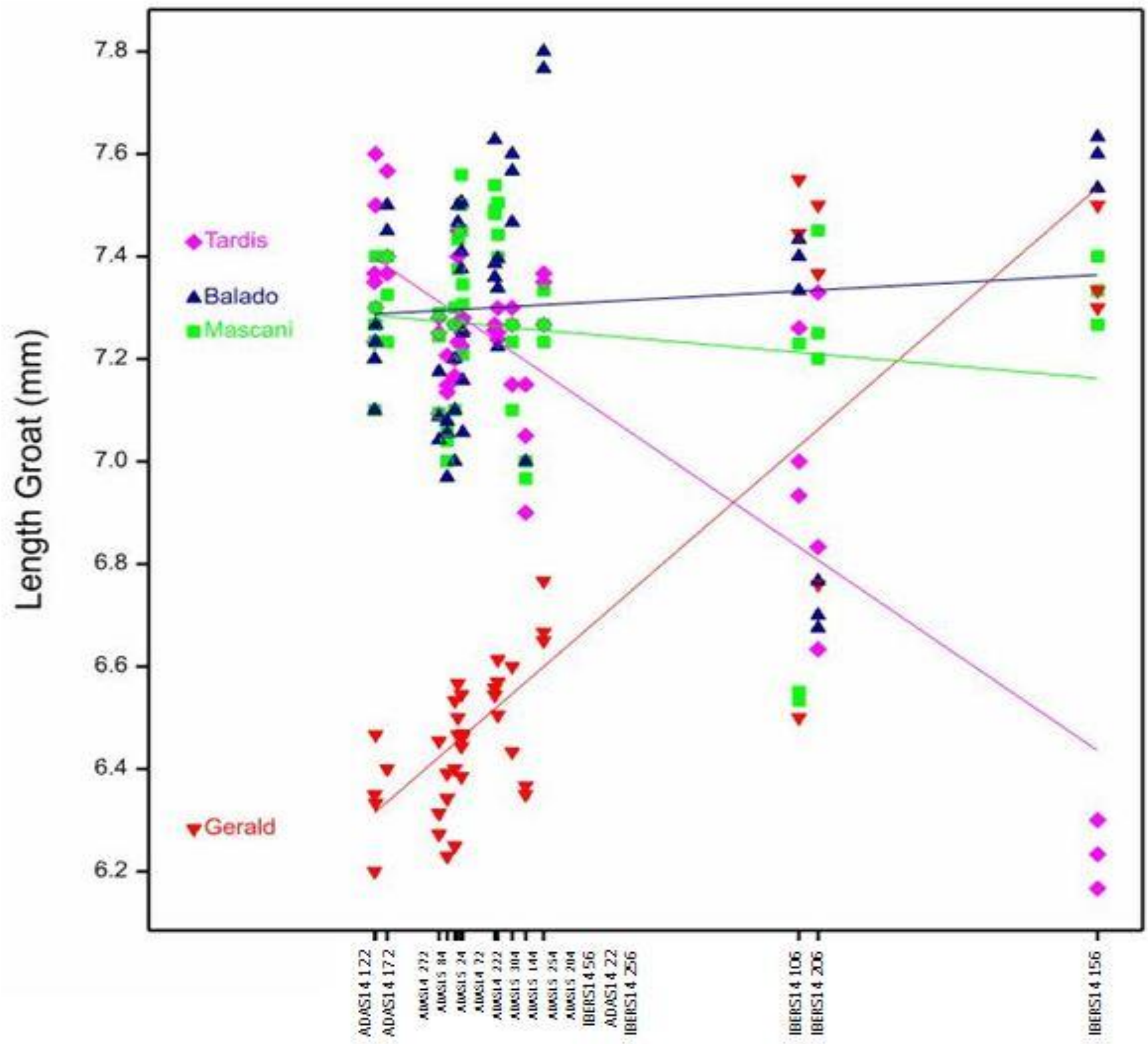


Figure 4.26 *Mean groat length (mm) ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at Rosemaund (ADAS) 2014, 2015 and IBERS 2015, harvest season.*

Table 4.23 *Mean groat length (mm) ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Groat Length (mm)	s.e.m.	Rank
IBERS 2014	156	7.49	0.196	1
IBERS 2014	206	7.29	0.119	2
IBERS 2014	106	7.27	0.137	3
IBERS 2014	256	7.09	0.143	4
ADAS 2014	22	7.07	0.110	5
IBERS 2014	56	7.06	0.137	6
ADAS 2015	204	7.05	0.129	7
ADAS 2015	254	7.05	0.143	8
ADAS 2015	144	7.03	0.126	9
ADAS 2015	304	7.03	0.162	10
ADAS 2014	222	7.02	0.145	11
ADAS 2014	72	7.02	0.130	12
ADAS 2015	24	7.02	0.124	13
ADAS 2015	84	7.01	0.136	14
ADAS 2014	272	6.97	0.148	15
ADAS 2014	172	6.96	0.131	16
ADAS 2014	122	6.96	0.130	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*

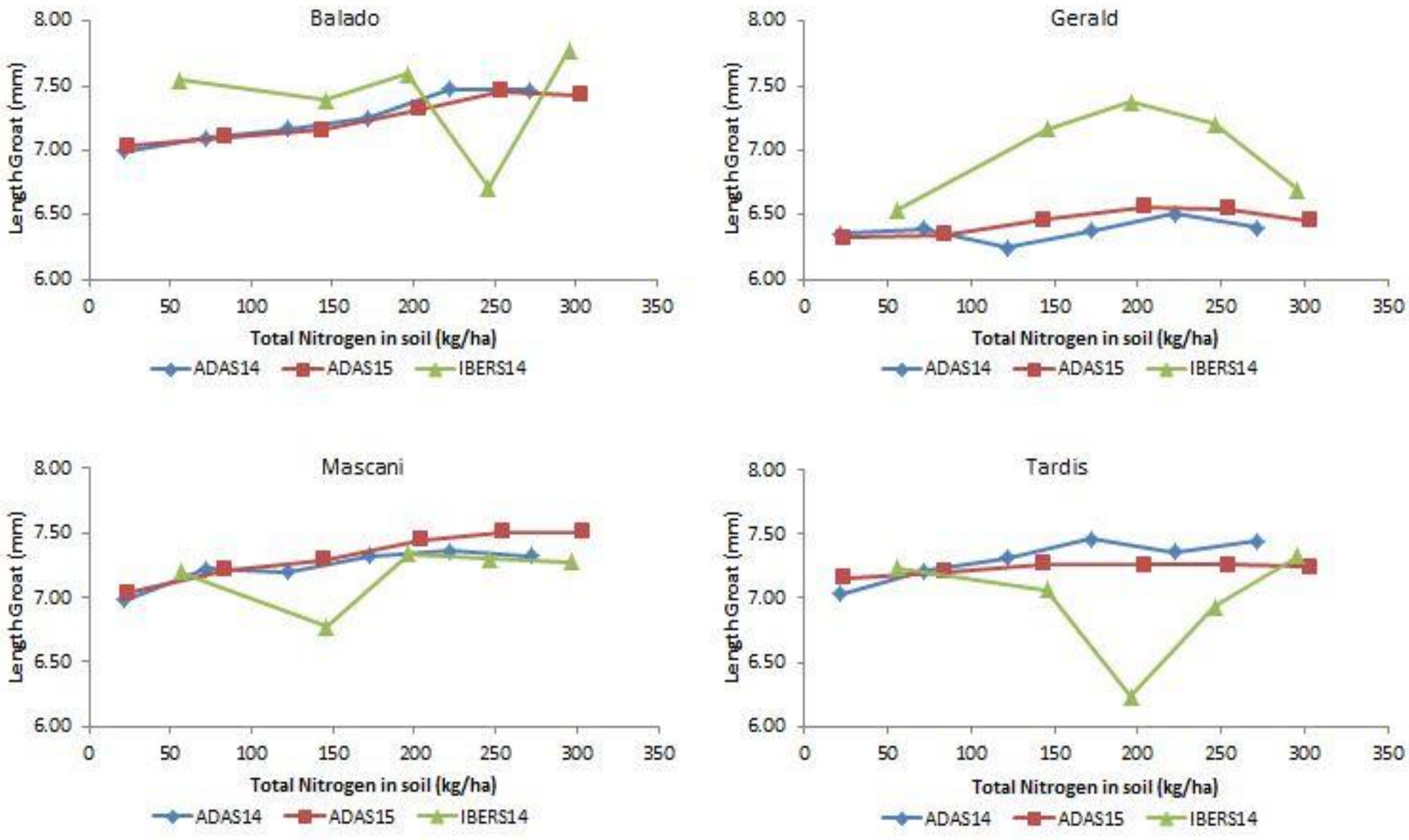


Figure 4.27 *Mean groat length (mm) ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at ADAS 2014, 2015 and IBERS 2014, harvest season.*

Mean groat length value by variety (figure 4.27) increased with higher levels of nitrogen fertilizer. This was particularly evident for Balado which had an increase in mean groat length at all sites, with higher levels of nitrogen fertilizer. This effect was highest at ADAS 2014. Mean groat length of Gerald did not change much, although at IBERS 2014 there was a 12.7% increase in length at level 2 in comparison to level 0. For Mascani the maximum response of mean groat length to nitrogen was at ADAS 2015 at both levels 4 and 5, whilst for Tardis, was at ADAS 2014 at level 5 of nitrogen fertilizer.

Joint regression analysis (figure 4.28, table 4.24) on groat ratio showed statistically significant differences in sensitivity values and total nitrogen levels (p-value<0.001). IBERS14 156 kg/ha total nitrogen showed higher groat ratio 0.40, whilst ADAS15 304 kg/ha total nitrogen; showed the lower, 0.34 (table 4.24). Gerald showed a lower sensitivity value, 0.56, and therefore higher stability against total nitrogen levels whilst Tardis with a sensitivity value of 1.54, showed lower stability against changes in total nitrogen levels.

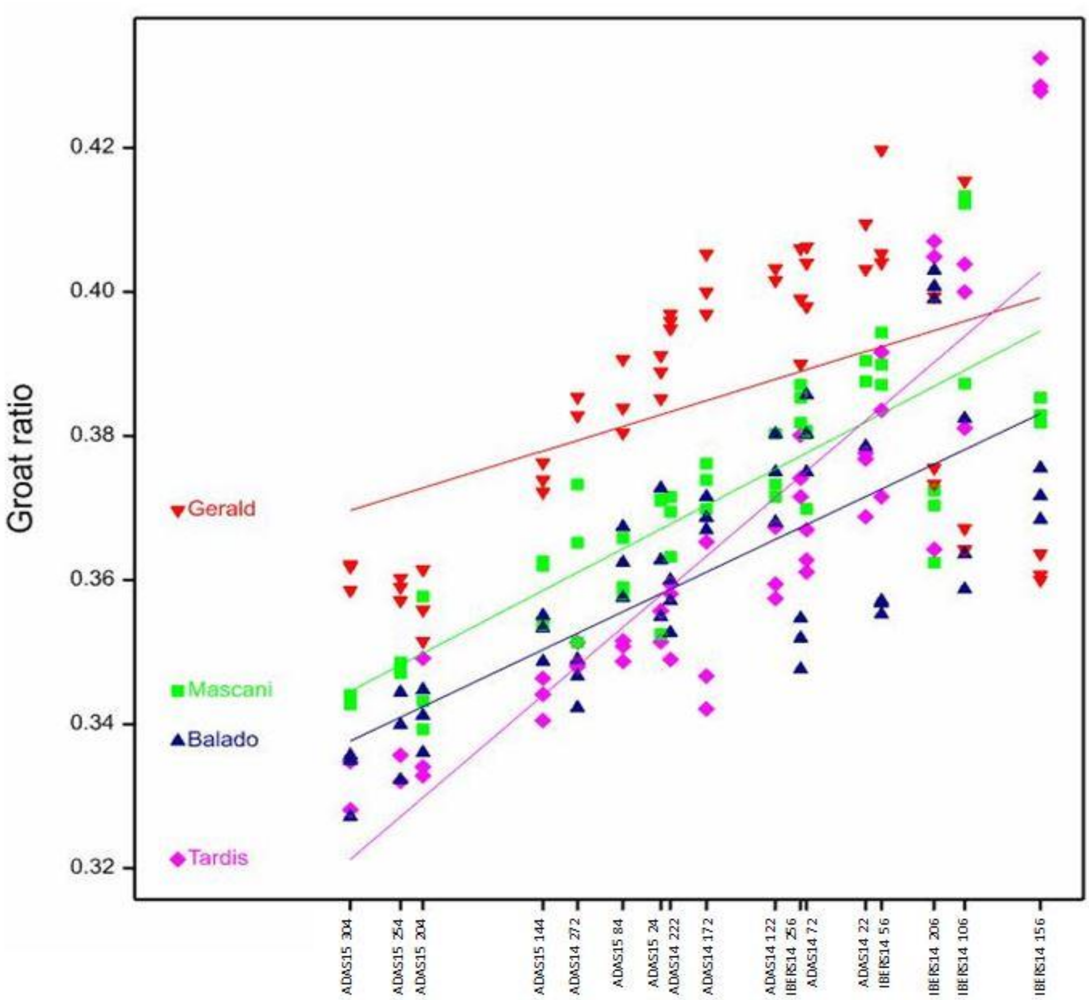


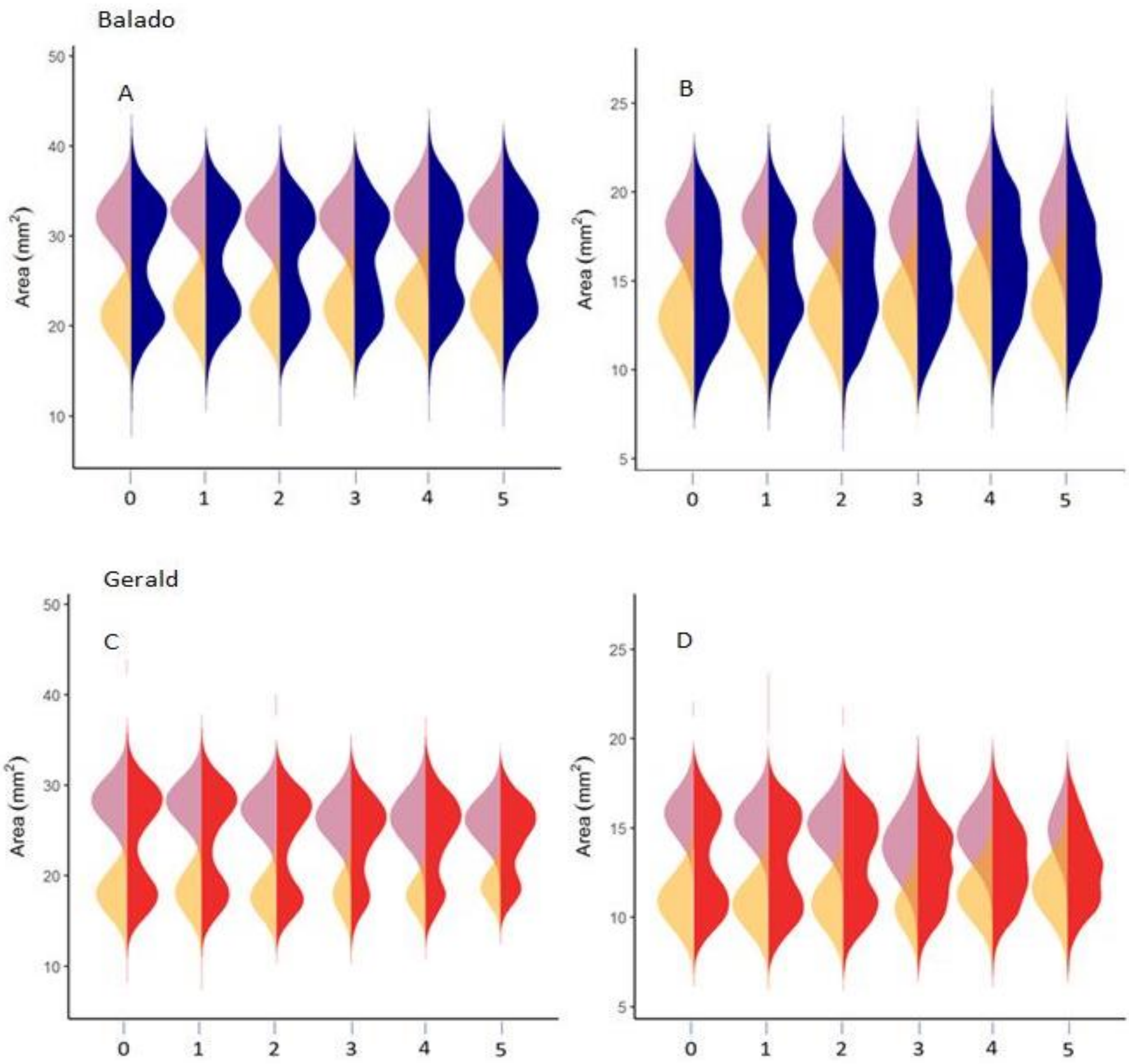
Figure 4.28 *Mean groat ratio ± s.e.m values by varieties at increasing levels of nitrogen applied (kg/ha) at ADAS 2014, 2015 and IBERS 2014, harvest season.*

Table 4.24 *Mean groat ratio ± s.e.m. and ranking from joint regression analysis at ADAS 2014 and 2015, and IBERS 2014 harvest season, by nitrogen level (see table 2.2.a, b) applied.*

Environment	Total Nitrogen (kg/ha) (SMN plus Nitrogen applied)	Groat Ratio	s.e.m.	Rank
IBERS 2014	156	0.40	0.010	1
IBERS 2014	106	0.39	0.007	2
IBERS 2014	206	0.39	0.006	3
IBERS 2014	56	0.38	0.007	4
ADAS 2014	22	0.38	0.028	5
ADAS 2014	72	0.38	0.021	6
IBERS 2014	256	0.38	0.357	7
ADAS 2014	122	0.38	0.018	8
ADAS 2014	172	0.37	0.021	9
ADAS 2014	222	0.37	0.022	10
ADAS 2015	24	0.37	0.005	11
ADAS 2015	84	0.36	0.005	12
ADAS 2014	272	0.36	0.027	13
ADAS 2015	144	0.36	0.004	14
ADAS 2015	204	0.35	0.003	15
ADAS 2015	254	0.35	0.004	16
ADAS 2015	304	0.34	0.005	17
Significance (G)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Significance (N)	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*
Sensitivities	*p-value<0.001*	*p-value<0.001*		*p-value<0.001*

4.3.7 Bimodality analysis

Frequency distribution analysis of the individual grain and groat data was conducted by site and season. The datasets were analysed to determine their bi-modality and to establish the mean, standard deviation and the numerical balance, i.e. proportion, between any subpopulations observed (Symons & Fulcher, 1988a). A bimodal frequency distribution was found regarding grain and groat area and length representing the primary and secondary grain found in each oat spikelet (figure 4.28) as also found in chapter three (see appendix for tables and graphs).



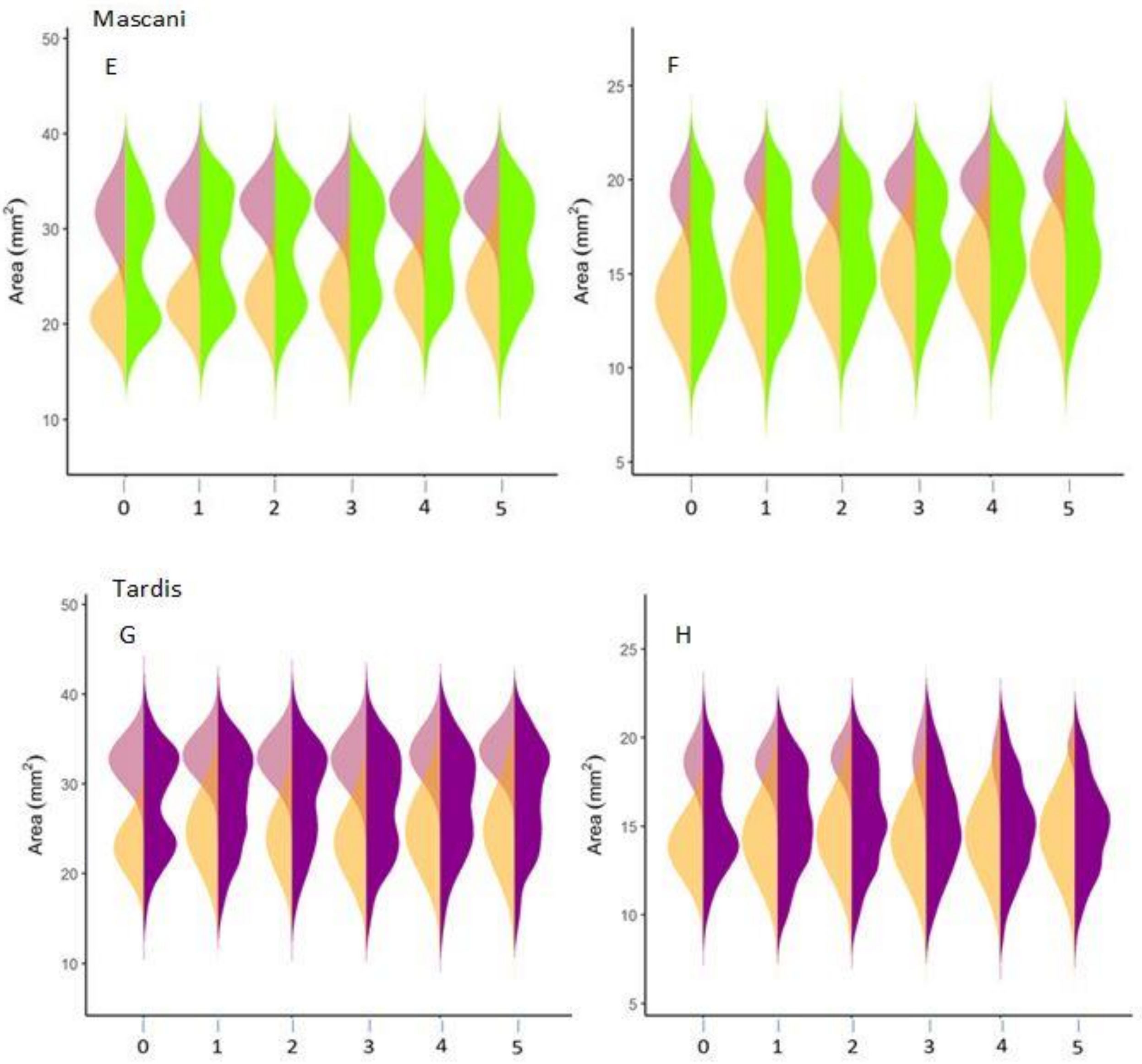


Figure 4.29. *Frequency of individual grain and groat area of Balado, Gerald, Mascani and Tardis grown at ADAS 2015 with increasing levels of nitrogen. A. Balado grain area; B Balado groat area; C Gerald grain area; D Gerald groat area, E Mascani grain area; F, Mascani groat area; G, Tardis grain area; H, Tardis groat area. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

An example of the bimodality distribution found in grain and groat area is represented in figure 4.28 A to H. These figures, called violin plots, show within each graph the effect of increasing levels of nitrogen on the proportion of the two subpopulations found when analysing frequency distribution parameters for grain and groat area of the four varieties at ADAS 2015. In all of them to the right are the distributions representing the classical frequency histogram. To the left are the fitted curves for the two subpopulations and (the overlap between them) with different colours for each subpopulation. From this analysis, the proportion of grain in each sub-population can be calculated along with other parameters of those sub-populations.

If we consider first the distribution of grain sizes (figure 4.29 A, C, E and G), as level of nitrogen applied increased, the separation between the two peaks in the bimodal distribution was less distinct and a greater overlap was found between them in all varieties except for Gerald. Increasing levels of nitrogen applied had a positive effect on the proportion of the secondary subpopulation (light orange curve in the graphs), increasing the overlap with the curve representing primary grain (light brown curve in the graphs), and therefore, the bimodality distribution was diminished with increasing levels of nitrogen (table 4.25). Gerald grain bimodality distribution analysis (figure 4.29 C), on the other hand, showed the opposite effect. Thus, increasing levels of nitrogen increased the proportion of the larger, primary grain, diminishing at the same time the proportion of secondary grain and therefore increasing the bimodality distribution found at higher levels of nitrogen applied.

The effect of increasing N on groat area frequency distributions was much more marked (figure 4.29 B, D, F and H) (table 4.25). In general, there was not such a clear distinction found between the sub-populations of groat sizes. This was more evident at higher levels of N applied with, for all varieties, the proportion of the smaller sub-population increasing at higher levels of N. This effect was particularly found for groats of Tardis at the higher N levels (figure 4.29 H and table 4.25) where only a small proportion of grain was classified into the primary sub-population.

Unlike the results for grain area, Gerald groat area (figure 4.29 D)) displayed the same effect when compared with the other three varieties, with a higher proportion of secondary groats with increasing levels of nitrogen and a bimodality diminished at higher levels of nitrogen applied (table 4.25). For these samples, a third sub-population of smaller grains was also detected.

Similar effects were found at IBERS 2014 and ADAS 2014 (see appendix for tables and figures).

Table 4.25 *Proportions, mean and standard deviations values, from each of the two sub-populations from the bimodal distribution analysis, by variety and at each level of nitrogen applied at ADAS 2015.*

ADAS 2015			Grain						Groats					
Variety	*Trait*	*N level*	*Proportion 2º*	*Proportion 1º*	*Mean 2º*	*sd 2º*	*Mean 1º*	*sd 1º*	*Proportion 2º*	*Proportion 1º*	*Mean 2º*	*sd 2º*	*Mean 1º*	*sd 1º*
Balado	Area mm^2	0	0.46	0.54	21.18	2.93	32.23	3.01	0.60	0.40	12.91	2.02	18.20	1.71
		1	0.52	0.48	22.12	3.16	32.88	2.70	0.64	0.36	13.59	2.08	18.63	1.53
		2	0.47	0.53	21.61	2.95	31.99	2.87	0.60	0.40	13.37	2.13	18.22	1.66
		3	0.49	0.51	22.03	3.19	32.11	2.95	0.56	0.44	13.41	2.07	18.24	2.01
		4	0.47	0.53	22.67	3.16	32.59	3.37	0.60	0.40	14.21	2.16	19.14	1.92
		5	0.51	0.49	22.47	3.39	32.42	3.10	0.56	0.44	13.67	2.08	18.39	2.09
Balado	Length mm	0	0.48	0.52	10.19	1.09	13.80	0.85	0.69	0.31	6.67	0.74	7.97	0.46
		1	0.55	0.45	10.59	1.21	14.10	0.77	0.72	0.28	6.77	0.71	8.05	0.46
		2	0.50	0.50	10.42	1.09	13.83	0.80	0.74	0.26	6.89	0.78	8.07	0.42
		3	0.52	0.48	10.68	1.16	14.03	0.88	0.77	0.23	7.04	0.76	8.37	0.40
		4	0.57	0.43	11.01	1.24	14.17	0.90	0.62	0.38	7.02	0.74	8.29	0.53
		5	0.64	0.36	11.16	1.36	14.46	0.76	0.73	0.27	7.12	0.71	8.40	0.49
Balado	Width mm	0	0.53	0.47	2.91	0.22	3.31	0.18	0.64	0.36	2.46	0.23	2.79	0.19
		1	0.29	0.71	2.82	0.19	3.24	0.20	0.77	0.23	2.53	0.23	2.83	0.14
		2	0.42	0.58	2.85	0.19	3.24	0.18	0.44	0.56	2.35	0.17	2.69	0.17
		3	0.82	0.18	2.99	0.26	3.27	0.15	0.45	0.55	2.33	0.21	2.65	0.19

		4	0.47	0.53	2.90	0.20	3.26	0.19	0.65	0.35	2.43	0.19	2.76	0.15
		5	0.63	0.37	2.91	0.23	3.23	0.18	0.58	0.42	2.36	0.22	2.66	0.17
Gerald	Area mm^2	0	0.45	0.55	18.04	2.47	28.33	2.59	0.58	0.42	10.89	1.47	15.82	1.31
		1	0.43	0.57	18.17	2.54	28.28	2.48	0.53	0.47	10.72	1.43	15.49	1.36
		2	0.40	0.60	17.64	2.25	27.39	2.50	0.48	0.52	10.77	1.44	15.24	1.39
		3	0.30	0.70	17.96	2.23	26.44	2.69	0.31	0.69	10.53	1.29	14.00	1.85
		4	0.26	0.74	17.81	1.89	26.49	2.95	0.48	0.52	11.32	1.49	14.68	1.63
		5	0.29	0.71	18.75	1.88	26.24	2.52	0.66	0.34	11.64	1.64	14.92	1.54
Gerald	Length mm	0	0.47	0.53	9.23	1.09	12.58	0.83	0.54	0.46	5.72	0.47	7.02	0.46
		1	0.45	0.55	9.30	1.09	12.56	0.74	0.52	0.48	5.74	0.52	7.04	0.41
		2	0.42	0.58	9.26	1.01	12.47	0.69	0.45	0.55	5.78	0.49	7.04	0.43
		3	0.35	0.65	9.55	1.02	12.43	0.78	0.43	0.57	6.03	0.53	6.99	0.56
		4	0.32	0.68	9.62	0.91	12.48	0.81	0.44	0.56	6.04	0.46	7.01	0.48
		5	0.35	0.65	9.82	0.93	12.48	0.79	0.68	0.32	6.15	0.52	7.15	0.43
Gerald	Width mm	0	0.67	0.33	2.81	0.21	3.18	0.14	0.78	0.22	2.39	0.21	2.76	0.10
		1	0.62	0.38	2.79	0.21	3.17	0.14	0.70	0.30	2.35	0.21	2.71	0.12
		2	0.69	0.31	2.76	0.22	3.10	0.15	0.76	0.24	2.35	0.20	2.66	0.12
		3	0.06	0.94	2.45	0.09	2.84	0.20	0.23	0.77	2.13	0.14	2.42	0.17
		4	0.03	0.97	2.36	0.05	2.83	0.21	0.75	0.25	2.31	0.19	2.52	0.16
		5	0.88	0.12	2.77	0.14	3.05	0.05	0.03	0.97	1.98	0.10	2.35	0.19
Mascani	Area mm^2	0	0.48	0.52	20.75	2.78	31.76	3.47	0.72	0.28	13.69	2.19	19.34	1.44

		1	0.47	0.53	22.04	2.92	32.70	3.19	0.78	0.22	14.71	2.60	19.99	1.22
		2	0.50	0.50	22.46	3.19	32.86	2.75	0.67	0.33	14.73	2.26	19.64	1.35
		3	0.51	0.49	23.05	3.54	32.70	2.90	0.67	0.33	15.00	2.36	19.80	1.32
		4	0.49	0.51	23.71	3.24	32.90	2.96	0.66	0.34	15.34	2.29	20.00	1.39
		5	0.57	0.43	23.80	4.05	33.06	2.88	0.76	0.24	15.56	2.52	20.23	1.26
Mascani	Length mm	0	0.55	0.45	10.41	1.17	13.76	0.85	0.76	0.24	6.74	0.71	8.07	0.35
		1	0.51	0.49	10.70	1.13	13.95	0.78	0.80	0.20	7.00	0.75	8.13	0.32
		2	0.58	0.42	10.86	1.34	13.95	0.85	0.76	0.24	7.07	0.73	8.16	0.32
		3	0.74	0.26	11.50	1.62	14.29	0.69	0.71	0.29	7.15	0.69	8.31	0.34
		4	0.70	0.30	11.63	1.47	14.34	0.76	0.72	0.28	7.25	0.71	8.35	0.33
		5	0.78	0.22	11.59	1.68	14.52	0.68	0.82	0.18	7.34	0.74	8.43	0.30
Mascani	Width mm	0	0.81	0.19	2.95	0.27	3.42	0.15	0.63	0.37	2.43	0.21	2.83	0.15
		1	0.60	0.40	2.92	0.21	3.35	0.16	0.60	0.40	2.47	0.22	2.83	0.15
		2	0.48	0.52	2.92	0.19	3.32	0.15	0.46	0.54	2.45	0.18	2.79	0.15
		3	0.73	0.27	3.03	0.24	3.32	0.12	0.59	0.41	2.48	0.22	2.77	0.13
		4	0.27	0.73	2.85	0.14	3.23	0.16	0.51	0.49	2.49	0.17	2.78	0.13
		5	0.75	0.25	3.03	0.25	3.29	0.12	0.66	0.34	2.50	0.22	2.76	0.13
Tardis	Area mm^2	0	0.49	0.51	22.95	3.13	32.76	2.80	0.70	0.30	13.86	1.85	18.65	1.38
		1	0.59	0.41	24.77	4.40	33.24	2.76	0.74	0.26	14.30	2.43	18.62	1.32
		2	0.51	0.49	24.02	4.04	32.99	2.88	0.76	0.24	14.52	2.42	18.89	1.24
		3	0.55	0.45	23.51	4.19	32.76	3.15	0.78	0.22	14.24	2.41	18.74	1.70

		4	0.64	0.36	24.84	4.79	33.38	3.02	0.88	0.12	14.54	2.50	18.91	1.42
		5	0.61	0.39	24.86	4.83	33.78	2.78	0.94	0.06	14.69	2.53	19.54	0.82
Tardis	Length mm	0	0.52	0.48	11.46	1.39	14.82	0.88	0.90	0.10	7.12	0.70	8.19	0.45
		1	0.67	0.33	12.16	1.84	15.00	0.79	0.08	0.92	5.78	0.32	7.38	0.67
		2	0.65	0.35	12.10	1.75	14.95	0.76	0.07	0.93	5.83	0.35	7.46	0.70
		3	0.71	0.29	12.04	1.89	14.98	0.98	0.03	0.97	5.56	0.25	7.37	0.77
		4	0.75	0.25	12.37	1.94	15.21	0.96	0.01	0.99	5.33	0.16	7.34	0.79
		5	0.75	0.25	12.60	1.99	15.39	0.84	0.08	0.92	5.96	0.44	7.43	0.73
Tardis	Width mm	0	0.49	0.51	2.88	0.18	3.21	0.15	0.85	0.15	2.51	0.20	2.80	0.10
		1	0.59	0.41	2.93	0.20	3.24	0.14	0.64	0.36	2.45	0.20	2.73	0.13
		2	0.33	0.67	2.83	0.17	3.17	0.16	0.55	0.45	2.40	0.18	2.69	0.13
		3	0.82	0.18	2.96	0.25	3.22	0.12	0.80	0.20	2.41	0.21	2.73	0.12
		4	0.07	0.93	2.52	0.16	3.02	0.20	0.07	0.93	2.06	0.11	2.47	0.19
		5	0.03	0.97	2.39	0.10	3.01	0.22	0.09	0.91	2.09	0.10	2.46	0.19

4.3.8 Correlations

Correlation analysis was done by site, i.e. ADAS 2014 and 2015, and IBERS 2014 harvest seasons and a two tail Pearson polynomial regression analysis, of physical and chemical quality traits and for grain and groat size and shape parameters. The three sites showed common significant (p-value<0.001) correlations with several grain and groat parameters and between physical and chemical quality traits.

Total nitrogen, i.e. the sum of nitrogen applied and nitrogen present in soil, had a positive correlation with yield kernel content, hullability, protein content and grain length at the three sites, showing increasing values of both traits with higher levels of nitrogen (table 4.26 and 4.27). At ADAS 2015 total nitrogen also displayed a positive correlation with hullability (%) but a negative correlation with groat ratio.

Yield (t/ha) was positively correlated (p-value<0.001) with protein content (%) at the three sites (figure 4.30). Polynomial regression analysis showed a significant curvilinear association with N levels, although the characteristic plateau was not reached.

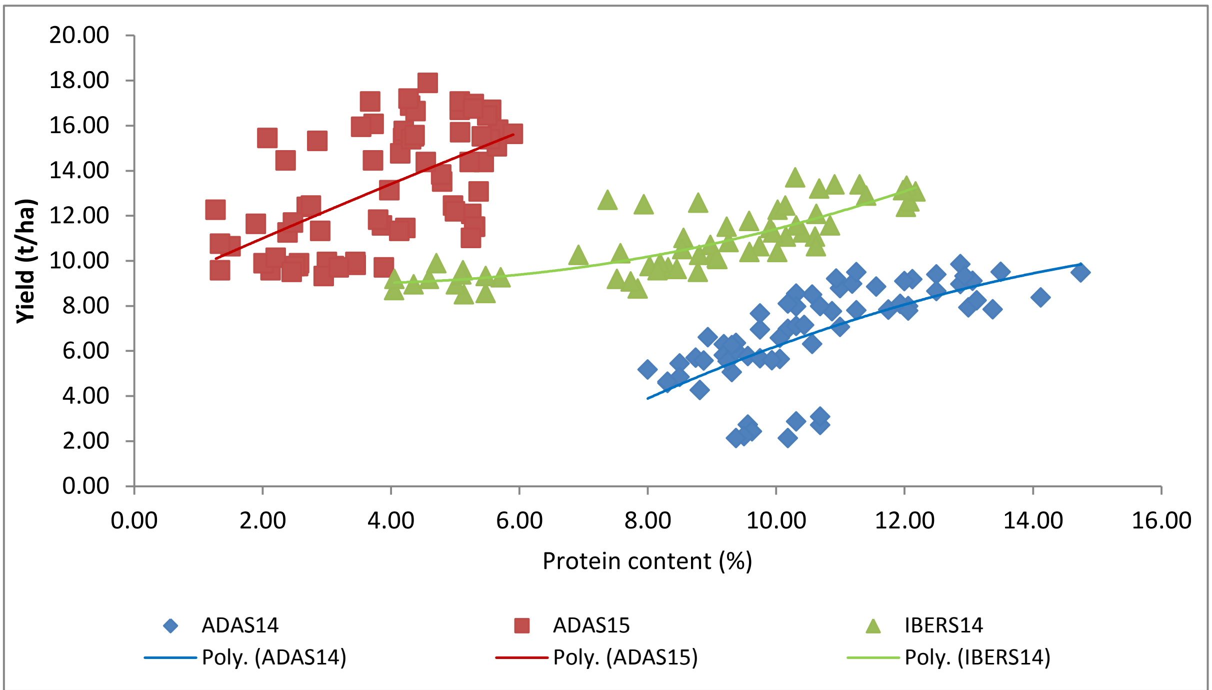


Figure 4.30 *Correlation plot between yield (t/ha) and protein content (%) values, of the three sites, ADAS 2014, 2015 and IBERS 2014, along with the curvilinear trend line between both parameters. Individual points are varieties by total nitrogen level, i.e. SMN and N applied.*

Specific weight displayed a significant strong positive curvilinear regression (p-value<0.001) and linear correlation coefficients with grain ratio for the three sites as shown in figure 4.31, table 4.27. All coefficients were similar in magnitude, although IBERS 2014 showed the highest correlation. Grain ratio is a measure of how round the grain is and this correlation suggests that rounder grain has a higher specific weight. Another trait that was positively correlated in the same curvilinear response with specific weight at all 3 sites (table 4.27) was a measure termed "grain density" (figure 4.32). This was calculated by dividing the grain TGW by the grain area and width. Specific weight was negatively correlated with grain length but this was only significant at 2 sites in 2014.

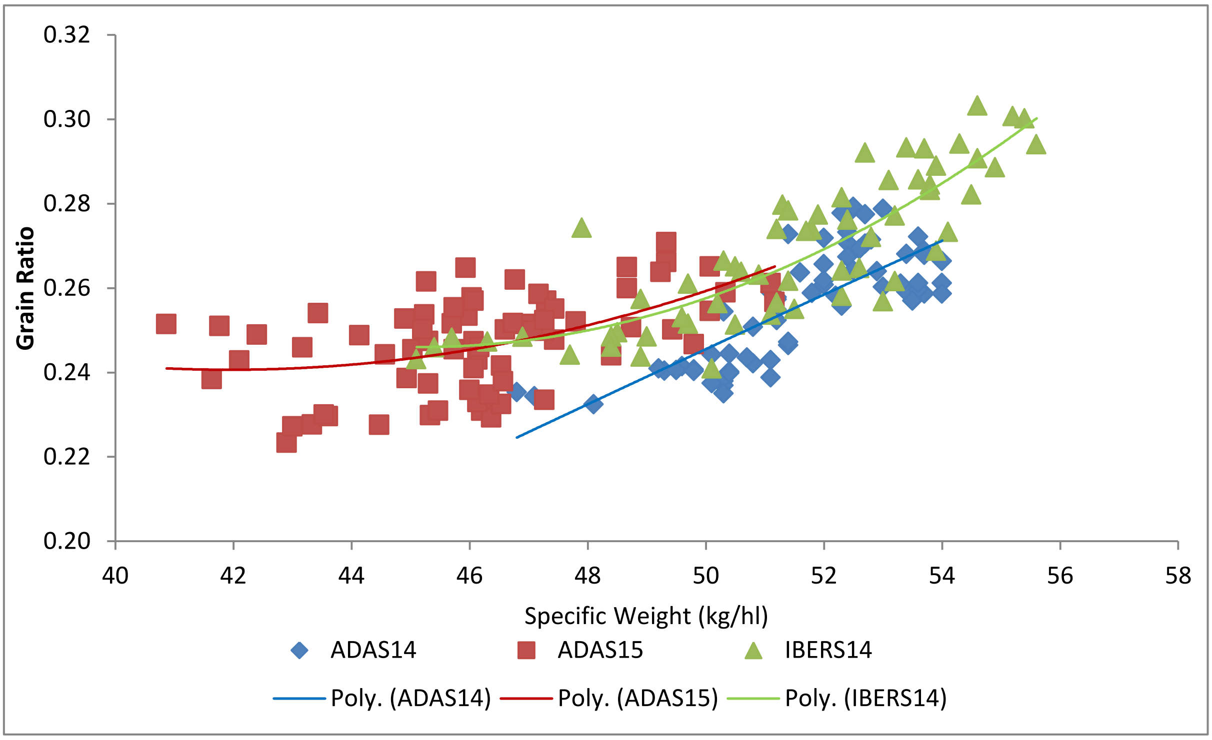


Figure 4.31 *Correlation plot between grain ratio and specific weight (kg/hl) values, of the three sites, ADAS 2014, 2015 and IBERS 2014, along with the curvilinear trend line between both parameters. Individual points are varieties by total nitrogen level, i.e. SMN and N applied*

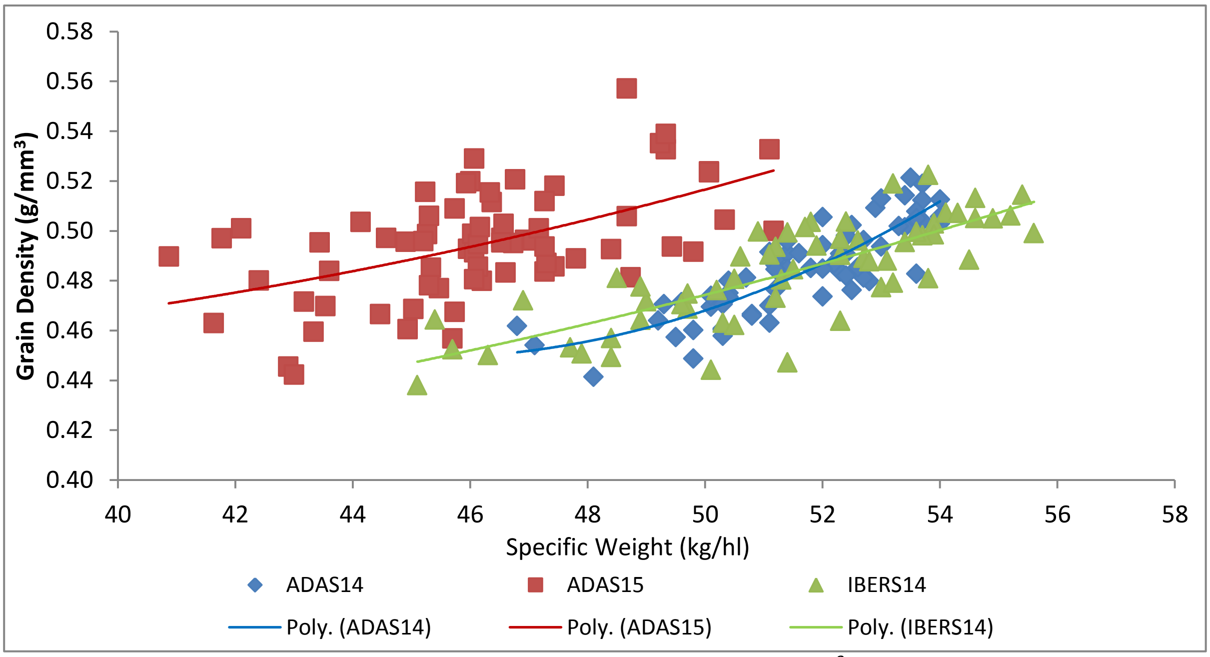


Figure 4.32 *Correlation plot between grain density (g/mm^3) and specific weight (kg/hl) values, of the three sites, ADAS 2014, 2015 and IBERS 2014, along with the curvilinear trend line between both parameters. Individual points are varieties by total nitrogen level, i.e. SMN and N applied*

No other correlations were found with specific weight that were consistent in more than one site were found. Thus, at IBERS 2014, a negative correlation was found between β-glucan content and grain area (mm^2) (table 4.27) with specific weight. At ADAS 2014, specific weight displayed a positive significant correlation with hullability (%) but a negative correlation with oil content (table 4.27), whilst at ADAS 2015 specific weight was also positively correlated with thousand groat weight, and at the same time positively correlated with kernel content, grain and groat density and groat width (table 4.27).

Kernel content was significantly positively correlated with hullability at all three sites. It was also negatively correlated with oil content at all three sites (figure 4.35) (table 4.27), showing a curvilinear regression. A positive correlation was also found between kernel content and grain density but this was only significant at the 2 sites in 2014. Positive correlations (table 4.27) between kernel content and thousand grain and groat weight, grain and groat width, groat area and groat density were only significant at ADAS 2014 and 2015.

Thousand grain weight at the three sites was strongly positively correlated (p-value<0.001) with grain width (mm) (figure 4.33) and was also significantly correlated with grain area (table 4.27). A curvilinear model regression analysis (figure 4.33) fitted better than a linear model when analysing the data. A positive correlation between thousand grain weight and grain length was found at IBERS 2014 and ADAS 2014 but this relationship was not significant at ADAS 2015. These data suggest that grain width is a greater determinant of thousand grain weight than grain length. All groat dimensions measured had significant positive associations with grain thousand grain weight (table 4.27). At ADAS 2014, thousand grain weight also displayed positive correlations with groat ratio and grain density (table 4.27). The latter correlation between grain density and thousand grain weight was also found at ADAS 2015 (table 4.27).

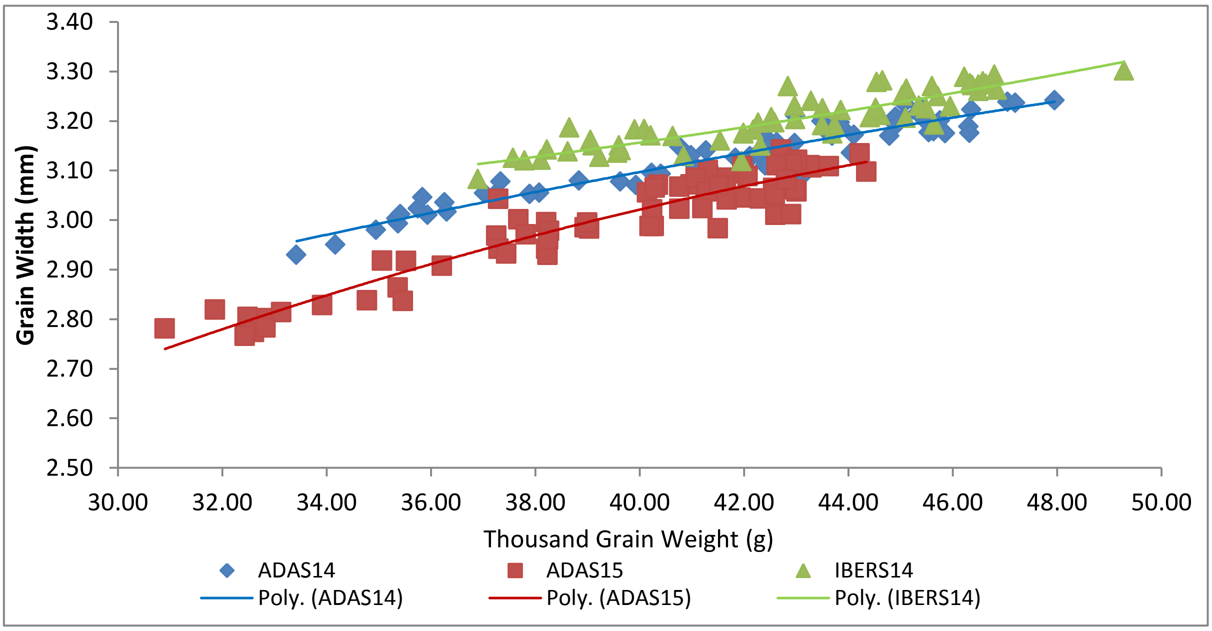


Figure 4.33 *Correlation plot between grain width (mm) and thousand grain weight (g) values, of the three sites, ADAS 2014, 2015 and IBERS 2014, along with the curvilinear trend line between both parameters. Individual points are varieties by total nitrogen level, i.e. SMN and N applied*

The only consistent significant correlation found for hullability, besides kernel content, was with oil content (table 4.27) (figure 4.34). At the three sites a significant (p-value<0.001) negative correlation was found between oil content and kernel content (figure 4.26) and hullability (figure 4.25). This negative correlation was stronger at ADAS 2015, in comparison with the other two sites correlation coefficients found. There were no significant correlations (p-value>0.05) hullability and any other physical nor chemical quality trait, or grain and groat size and shape parameters (table 4.27). ADAS 2014 hullability was positively correlated with grain and groat density with a correlation coefficient of 0.62 and 0.64 respectively at IBERS 2014 and ADAS 2015 (table 4.27).

Chemical traits showed different significant correlations (p-value<0.001) with several grain and groat size and shape traits when analysed by site (table 4.26) but no consistent significant results were found across all three sites.

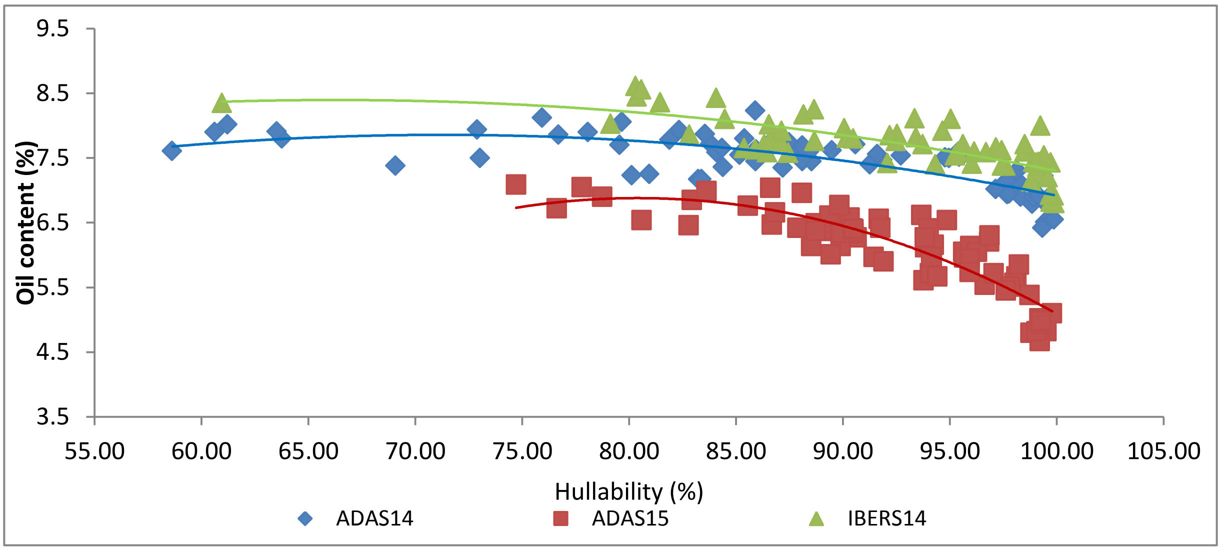


Figure 4.34 *Correlation plot between oil content (%) and hullability (%) values, of the three sites, ADAS 2014, 2015 and IBERS 2014, along with the curvilinear trend line between both parameters. Individual points are varieties by total nitrogen level, i.e. SMN and N applied*

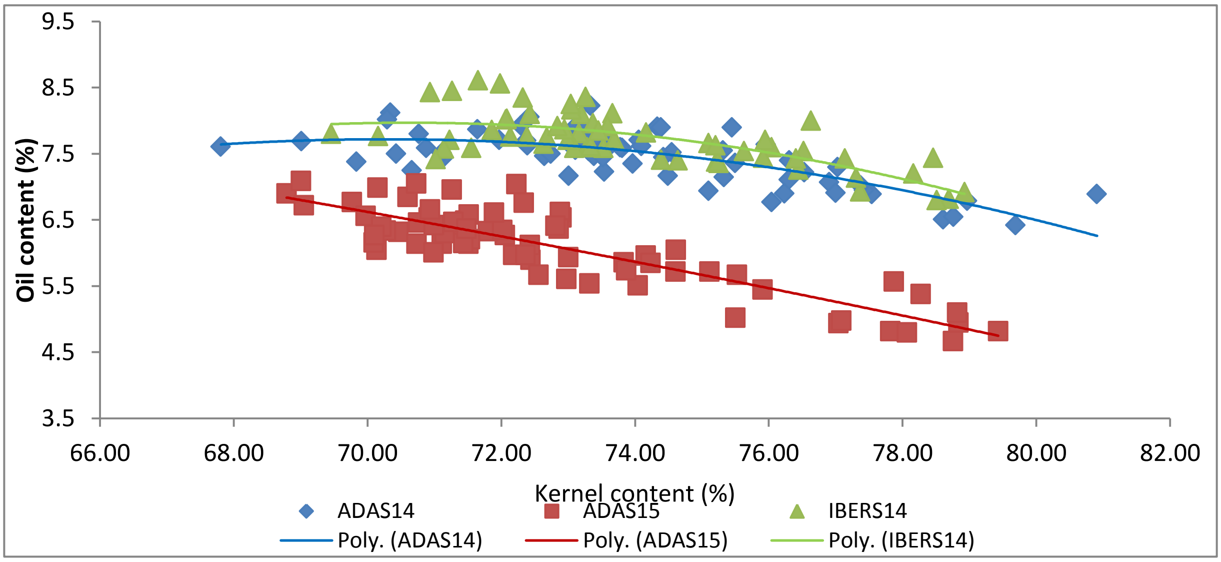


Figure 4.35 *Correlation plot between oil content (%) and kernel content (%) values, of the three sites, ADAS 2014, 2015 and IBERS 2014, along with the curvilinear trend line between both parameters. Individual points are varieties by total nitrogen level, i.e. SMN and N applied*

For example, at ADAS 2014 oil content was a significantly (p-value<0.001) negatively correlated with thousand groat weight, whilst at ADAS 2015 a negative correlation was found between oil content and groat area (table 4.26).

Protein content correlations with groat ratio by site were significantly negative (p-value<0.001) (table 4.26) at ADAS 2014 and 2015. At the same time, at ADAS 2015 a negative correlation was found between protein content and oil content (figure 4.36) and a positive correlation with β-glucan content and hullability (table 4.26).

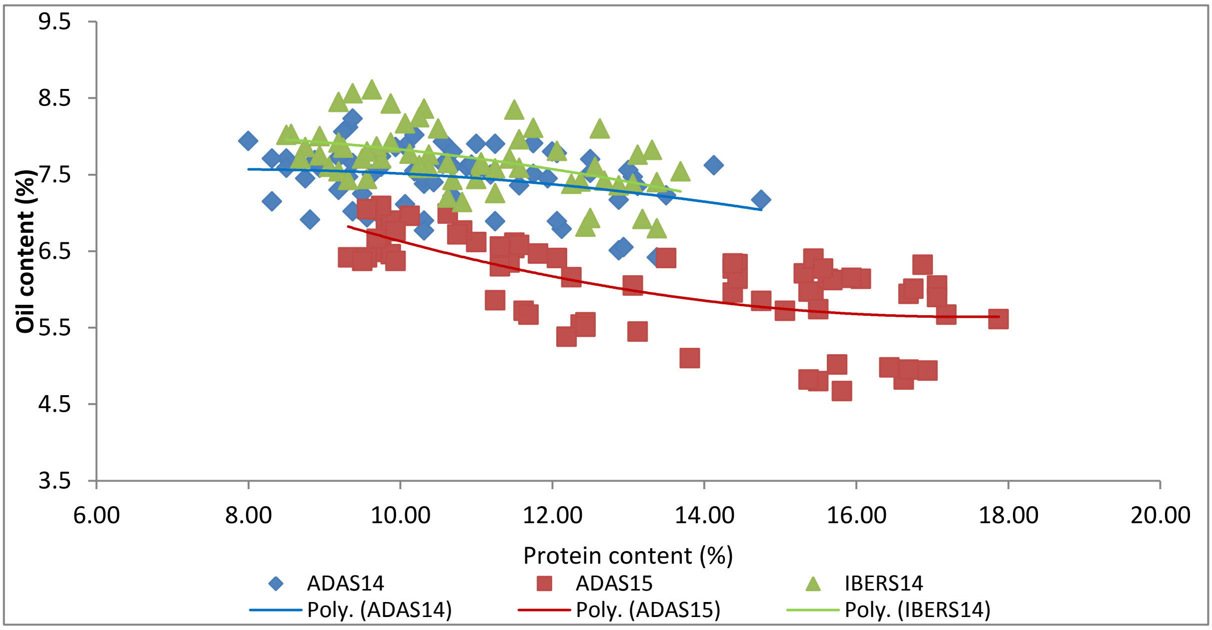


Figure 4.36 *Correlation plot between oil content (%) and protein content (%) values, of the three sites, ADAS 2014, 2015 and IBERS 2014, along with the curvilinear trend line between both parameters. Individual points are varieties by total nitrogen level, i.e. SMN and N applied*

β-glucan content was positively correlated with thousand grain weight but this was only significant at IBERS 2014 and ADAS 2015. No other significant (p-value>0.05) correlations were found between β-glucan content at ADAS 2014 with any trait. However, there were significant positive correlations (p-value<0.001) with grain area and length and negative correlations with grain ratio at IBERS 2014. At ADAS 2015 β-glucan content showed the same positive correlations with groat area and length, negative correlations with groat ratio and a positive correlation with hullability (p-value<0.001) (table 4.26).

Table 4.26 *Protein, oil and β-glucan content linear correlation coefficients (p-value<0.001) with grain and size traits at ADAS 2014 and 2015 and IBERS 2014. Only significant correlation coefficients above or below ± 0.55 (threshold for interpretation) are displayed.*

	ADAS 2014	***ADAS 2015***	***IBERS 2014***	***ADAS 2014***	***ADAS 2015***	***IBERS 2014***	***ADAS 2015***
	Protein			*Oil*		*β-glucan*	
Protein	1.00	1.00					
Oil		-0.64	1.00	1.00	1.00		
β-glucan		0.55				1.00	1.00
Area Grain						0.73	
Length Grain						0.69	
Grain Ratio						-0.64	
Grain Density				-0.72			
TGW Groat				-0.57			
Area Groat					-0.55		0.60
Length Groat							0.68
Groat Ratio	-0.57	-0.66					-0.65
Groat Density				-0.74	0.62		

Table 4.27. *Nitrogen total (SMN and N applied), yield (t/ha), specific weight (kg/hl), kernel content (%) and hullability (%) Pearson linear correlation coefficients (p-value<0.001) of each parameter at ADAS 2014 and 2015 and IBERS 2014. Only coefficients above or below ± 0.55 are coloured in red and green respectively.*

	IBERS 2014	**ADAS 2014**	**ADAS 2015**	**IBERS 2014**	**ADAS 2014**	**ADAS 2015**	**IBERS 2014**	**ADAS 2014**	**ADAS 2015**	**IBERS 2014**	**ADAS 2014**	**ADAS 2015**	**IBERS 2014**	**ADAS 2014**	**ADAS 2015**	**IBERS 2014**	**ADAS 2014**	**ADAS 2015**
	N Total			*Yield*			*Sp-Wt*			*Kernel content*			*Hullability*			*Thousand Grain Weight*		
Yield	0.90	0.89	0.71															
Protein	0.93	0.84	0.93	0.79	0.68	0.58												
Oil								-0.65										
β-glucan							-0.71									0.55	0.71	
Kernel content									0.65								0.60	0.61
Hullability			0.61					0.80		0.69		0.73						
TGW											0.61	0.62				1.00	1.00	1.00
Area Grain							-0.72									0.76	0.83	0.76
Width Grain												0.57				0.83	0.95	0.90
Length Grain							-0.79	-0.64								0.59	0.61	
Grain ratio							0.82	0.78	0.76					0.57				
Grain Density							-0.69	0.69	0.67	-0.60	0.64			0.62			0.72	0.81

TGW							0.56		0.69	0.73				0.81	0.97	0.96
Groat Area									0.57	0.68				0.83	0.93	0.94
Groat Width							0.61			0.58				0.73	0.85	0.88
Groat Length										0.56				0.71	0.83	0.82
Groat Ratio	-0.63														-0.58	
Groat Density							0.63		0.64	0.80		0.64			0.71	0.81

4.4 Discussion

Oats are a low input cereal and so are believed to need lower fertilization levels in comparison with other cereals, e. g. wheat and barley (Kindred *et al.*, 2008). The effects of fertilization level applied during oat yield development on grain quality parameters have been so far poorly understood in comparison with wheat and barley. This is due to, on one hand, less research on investigating influence of nitrogen levels of fertilization on milling industry grain and groat quality parameters, despite the recent increase of interest of oats for human consumption (Bennet, 1989; Abdel-Aal & Wood, 2004; Wrigley, 2010) and on the other hand due to a focus on the effects of nitrogen on oat yield and lodging (Zhou, Kumar Biswas & Ma, 2013).

Optimum fertilization levels are one of the main management tools in oats to enhance its competitiveness among other cereals. Oats due to its long stems, are considered prone to lodging, which might result in a loss in yield and grain quality particularly at high levels of N application (Chalmers et al., 1998). It is crucial to obtain the maximum yield and grain and groat quality to maximize milling industry and farmer benefits, minimizing at the same time lodging, the cost to the producers and environmental impacts.

The strategy for breeders is to develop varieties with better yield and stability, avoiding lodging that may cause losses. Nitrogen has been shown, in this thesis, to increase yield, with statistically significant differences between varieties and levels of nitrogen in all sites and showing a strong positive correlation with higher levels of nitrogen, as previously reported (Frey, 1959; Brinkman & Rho, 1984). This positive effect was particularly high at IBERS 2014 in comparison with a more moderate effect at ADAS 2014 and 2015. The same result was observed in the varieties, with much higher values obtained at IBERS 2014 level 4, when compared to level 5 at ADAS 2014 and 2015. No differences were observed between varieties within a site, with all displaying similar increases with higher levels of nitrogen.

A strong positive correlation was found between protein content and yield, both showing a significant positive response with increasing levels of nitrogen applied, as previously reported (Welch & Leggett, 1997; Chalmers et al., 1998). Protein content at all sites reflected the same positive effect, being highly significant at ADAS 2015, level 5 with

49.9% more protein content in comparison to level 0. Despite non-significant differences found in the response to nitrogen fertilization between varieties in protein content, a positive effect was observed for all of them. These results support increasing nitrogen levels of fertilization to increase yield without compromising levels of protein content under milling quality requirements and standards.

β-glucan content displayed similar results to protein content with significant higher values with increasing levels of nitrogen, particularly at ADAS 2014 with 14.9% more β-glucan when compared to level 0. Joint regression analysis showed significant differences in variety sensitivity values, meaning that although there were no differences in b-glucan content by varieties, the adaptability to changes in the environment was different between those varieties.

Oil content on the other hand, in a mirror effect with protein content, showed diminishing values with increasing levels of nitrogen fertilizer, reaching the lowest content at level 5 at all sites. This diminishing effect was particularly significant at ADAS 2015 with 13.5% lower oil content in comparison with level 0. Also significant differences between varieties were found, although no interaction between nitrogen and genotypes. However, the same effects described above have been observed when analysing the effect of higher nitrogen levels on varieties' oil content. As previously reported (Welch & Leggett, 1997) although there was a slight increase on oil content with lower levels of nitrogen there was a final diminishing effect at the highest levels, being Mascani the most affected with 7% reduction. Negative significant correlations were found between oil content and kernel content and hullability at all sites. Although previous research has reported a negative correlation between oil content and protein content (Welch & Leggett, 1997), this effect was only found at ADAS 2015, which might explain the mirror effect previously mentioned.

In this study, all milling quality traits displayed a significant curvilinear response to total nitrogen level. However, the classical plateau was not reached suggesting that higher levels of n might be applied to look for the optimum rate of fertilizer. Specific weight was, in accordance with previously reported results (Ohm, 1976; Givens, Davies & Laverick, 2004), lower with higher levels of nitrogen at all sites, although this was variable from site to site. Thus, at ADAS 2014 and 2015 a slight increase in specific weight values was observed at lower levels of nitrogen. Incomplete grain filling and therefore less dense grains, due to competition because of an increased shoot number have been previously found to be correlated with higher levels of nitrogen applied (Chalmers et al., 1998; Browne et al., 2004; Muurinen, Slafer & Peltonen-Sainio, 2006), and this might explain lower specific weight values. At the same time increased levels of nitrogen resulted in higher values of grain length but lower values of grain width, i.e. longer and thinner grains, that along with poor grain filling, might contribute to hulls being more loosely attached to the width groat and therefore diminishing specific weight. On the other hand, positive correlations were found between specific weight and grain ratio and grain density at all sites and negative correlations with length grain but only at IBERS 2014 and ADAS 2014. As a packaging character, specific weight is the weight of grains which fills a specified volume under standard packing conditions. According to the correlations found in this thesis, the lower specific weight might be due to higher effect of the grain ratio which shows lower values with higher levels of nitrogen applied, with length greater with higher levels of nitrogen applied, than due to the effect of nitrogen increasing grain density.

Higher levels of nitrogen had also a positive effect on kernel content and hullability, with significant differences between varieties and levels of nitrogen and interactions between the two factors for both kernel content and hullability. ADAS 2014 showed the higher response for both kernel content and hullability with higher levels of nitrogen. A significant positive correlation was found between the two quality parameters at all sites. Only Mascani with very high values of hullability at level 0 of nitrogen showed a small response to nitrogen applied, whilst the rest of varieties had 4% increases in kernel content and 35% average increases in hullability with higher levels of nitrogen.

Significant differences were found between nitrogen levels and varieties when analysing thousand grain weight. However, levels of nitrogen proved to have a non-consistent effect on thousand grain weight at the three sites and between varieties. Thus, at ADAS 2014 and IBERS 2014 the largest grains were obtained at lower levels of nitrogen, whilst at ADAS 2015 grains were heaviest at level 5. On the other hand the strong positive correlation found between thousand grain weight and grain width explains the similarity found when analysing the effect of nitrogen on grain width, with similar increases effect at lower levels of nitrogen at ADAS 2014 and IBERS 2014 and a higher increase at ADAS 2015 at higher levels of nitrogen. On the other hand grain and groat size parameters were also positively affected by increasing levels of nitrogen applied. However, none of the size parameters reached the higher levels with the highest total nitrogen level at ADAS 2015 304 kg/ha but at IBERS 2014 256 kg/ha. Tardis showed the lower sensitivity values for groat area, width and length and whilst Gerald showed the higher for the same groat size parameters. This suggests that Tardis groat size and shape is more stable to changes in the environment including changes in total nitrogen levels.

Analysis of grain and groat frequency values resulted in bi-modal distributions for grain and groat area and length, reflecting the primary and secondary grain found in each spikelet. At the same time, positive effects on grain and groat area and length for both primary and secondary grain, with higher values with increasing levels of nitrogen, were found. On the other hand, increasing levels of nitrogen resulted in a diminishing effect on the bi-modal character of those distributions. The overlap that allows differentiating between primary and secondary grain and groat area and length increased, having as a consequence the homogenization of the proportion of primary and secondary grain and groat being more evident in groats. For varieties, the same effect was found when analysing the proportion of grain and groat under each sub-population, although in a different way. Balado, Tardis and Mascani increased the proportion of secondary grain and groat whilst Gerald showed this effect on primary grain and groat proportion.

In this chapter, the effect of different levels of nitrogen on grain and groat samples from two seasons and three sites, regarding grain and groat size and shape and quality parameters was analysed. Despite a negative effect on specific weight, the main quality parameter for the milling industry and producers, and a variable effect on thousand grain weight, the levels of nitrogen applied had positive effects and did not reach a plateau for yield, protein content, kernel content nor hullability. Sensitivity values showed differences between varieties for hullability, oil content and β-glucan content, in their stability to changes in the environment, i.e. changes in N level. The non-significant interaction between kernel content and hullability with nitrogen levels suggests that N has an influence on variety selection when breeding to enhance both quality parameters. On the other hand, the negative effect on oil content and the enhanced protein and β-glucan content might result in a better chemical composition in terms of human consumption, for the milling industry. The loss of bimodality in grain and groat area and length might also benefit the milling industry, which establishes the downstream oat processing according to the size and shape of grain and groats.

Statistical relationships found between certain quality parameters and nitrogen levels of fertilization were curvilinear regressions. The search for an optimum nitrogen rate or at least the plateau above which increasing levels of nitrogen fertilizer have no benefits for grain quality parameters remains unclear. It might require on one hand, further research with intermediate quantities of nitrogen to apply as fertilizer, taking into account results found in this thesis, and on the other hand a better understanding of the relationships that can be established between grain and groat size and shape and grain quality parameters.

4.5 Conclusions

- Increasing levels of nitrogen had a negative effect on specific weight, the main quality parameter for the milling industry and producers, and a variable effect on thousand grain weight. However, it had positive effects on yield, hullability, kernel content, protein and β-glucan content.
- Total nitrogen, i.e. nitrogen in soil (SMN) and applied, did not reach a plateau for any of the milling quality parameters and had in all cases a higher effect, neither positive nor negative, on milling quality parameters. However, despite it was at ADAS 2015 where higher levels of nitrogen were applied it was at IBERS 2014, with lower maximum levels of total nitrogen, where yield, kernel content, thousand grain weight, hullability and oil content were higher.
- Varieties were positively affected by increasing levels of total nitrogen in soil, i.e. SMN and nitrogen applied, in yield, kernel content, hullability, and protein and β-glucan content. On the other hand, varieties specific weight and oil content were negatively affected by increasing levels of total nitrogen, whilst varieties thousand grain weight showed variable results. These results were in accordance with results found when analysed by nitrogen levels.
- Variety sensitivities values for each quality parameter analysed were not always significantly different between varieties. This means that the differences found in yield, specific weight, kernel content and protein content cannot be predicted based on the

adaptability or stability to changes in the environment of each variety. However, variety hullability, oil content and β-glucan content did show significant differences in sensitivity values, and therefore the regressions provided a means of predicting relative performance under changing environmental conditions, i.e. total nitrogen.

- Grain and groat size parameters were positively affected by increasing levels of nitrogen. However, higher levels of total nitrogen did not yield higher grain and groat size values. Thus, IBERS 2014 256 kg/ha and 106 kg/ha showed the higher values of area and width, whilst ADAS 2014 222 kg/ha had the higher grain length. Groat size parameters were consistently higher at IBERS 2014, but no at the higher levels of nitrogen but intermediate.
- Tardis sensitivity values in groat size parameters were consistently lower in area, width, length and ratio suggesting higher stability and therefore relatively predictable responses to the changes in the environment.
- Increasing levels of total nitrogen affect bimodality distribution of the two grain subpopulations, i.e. primary and secondary grain, for each variety.
- The no statistically significant interaction between total nitrogen levels and variety suggest higher influence of variety when breeding for enhancing milling quality parameters, i.e. kernel content and hullability.
- The search for an optimum nitrogen rate or at least the plateau above which increasing levels of nitrogen fertilizer have no benefits for grain quality parameters remains unclear.

Chapter five. Grain development

5.1 Introduction

One of the most important aspects, agronomically speaking, in grain quality is to establish the moment of maximum grain growth so as to harvest oats when it is most suitable in terms of quality parameters for the milling industry, therefore yielding maximum benefit. These include kernel content, specific weight, thousand grain weight, oil, protein and β-glucan content and moisture content.

Harvest date however is often determined by factors such as weather conditions, to avoid possible diseases, weeds and insects. For example, if oats are left to dry down in the field they can deteriorate, the surface of the kernel may be attacked by a fungus and discolour or turn black. This is undesirable as dark kernels are unacceptable for milling, thus, oats should be combined as soon as they are ripe with preferably a moisture content of approximately 14%. Understanding when maximum grain quality is reached for the milling industry could benefit producers and end-users enabling the highest standards of grain and groat quality parameters.

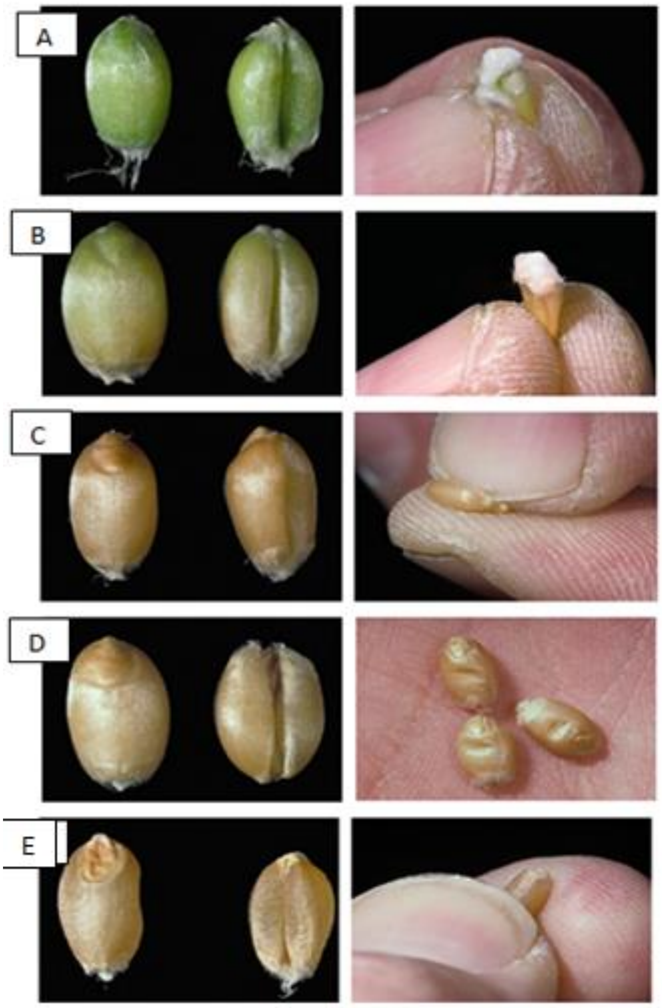


Figure 5.1 *Grain filling stages in wheat. Image extracted from* Mukherjee et al., 2015. *A: Early milk; B: Late milk; C: Soft dough; D: Hard dough; E: Ripening.*

In crops such as wheat and barley, we can differentiate several stages during grain development (figure 5.1 from Mukherjee , Liu, Deol, Kulichikhin, Stasolla, Brule-Babel & Ayele, 2015). For wheat and barley these have been described as follows:

✓ *Early milk (A)*: The grain contains white, watery liquid

✓ *Medium milk*: The grain is nearly full length and contains a soft wet centre in watery liquid. The grain can be squeezed from between lemma and palea.

✓ *Late milk (B)*: The grain contents are wet and sticky when crushed.

✓ *Early dough*: The grain contents are soft and cheesy.

✓ *Soft dough (C)*: The grain contents are firm and not easily squeezed out. A finger nail quickly disappears. The grain has reached maximum fresh weight and contains approximately 50% moisture. The green colour is fading.

✓ *Hard dough (D)*: The grain contents are dry and cannot be squeezed out. A finger nail impression remains. Maximum dry weight has been reached and the grain contains approximately 30% moisture.

✓ *Ripening (E)*: The grain is fully mature and hard to the touch.

In oats so far, there is no description of the several stages in grain development. The inflorescence in oats (figure 5.2) (National Institute of Agricultural Botany (Great Britain), McGarel 2000) is called a panicle and differs from wheat and barley in which the inflorescence is a spike. The panicle consists of a main stem bearing whorls which at the same time bear branches. At each branch are found the spikelets. Inside each spikelet two to three grains develop, differentiating into primary, secondary and tertiary grain, each of them protected by a leaf like structure, the husk, composed of a lemma and a palea.

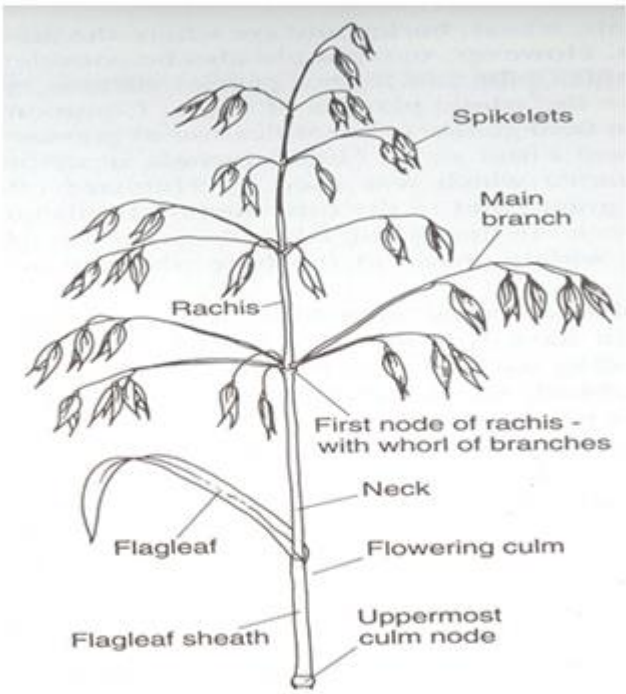


Figure 5.2. *Structure of a panicle in oats. The characters used as cultivar descriptors are showed*(National Institute of Agricultural Botany (Great Britain), McGarel, & Ardies, 2000).

This panicle architecture in oats has implications during grain filling due to differential distribution of photosynthate (Browne et al., 2006). As a result of these and along with a wide range in flowering time across the panicle (Griffiths 2010), differences in grain development between the top and the bottom of the panicle's spikelets are found resulting in a mix of completely and not completely mature grain at final harvest. It has been reported that primary grain is usually larger than the secondary grain but with a lower kernel content and hullability when compared to secondary grain (Browne et al., 2002). These differences in grain size and shape parameters also determine the bimodal distribution previously reported (Symons & Fulcher, 1988.b). This mixture of grain types may decrease the specific weight and kernel content, increasing the presence of screenings and affect the hullability and thousand grain weight values (Browne et al., 2006). Moisture content is also thought to have an important role in hullability of the grain. During the ripening of the grain, moisture content decreases, allowing the kernel, to separate from the lemma and palea, i.e. the husk, thus increasing hullability and milling quality of the cultivated oat (Browne et al., 2002; White & Watson, 2010).

Although some similarities can be found between oats, barley and wheat, the panicle architecture of oats described above justifies a deeper study on grain development in oats. These developmental stages have not been studied before in terms of changes in grain size and shape that may affect and influence kernel content, thousand grain weight and other milling quality parameters. By the study of the variation in grain and groat shape, i.e. grain area, length and width, we can understand the different stages of development inside the spikelet among primary, secondary and tertiary grain, and between varieties, allowing us to establish the best conditions for grain development to get the best and highest quality parameters.

Given oat panicle peculiarities three main objectives were therefore investigated in this research. Firstly, to analyse kernel content, thousand grain and groat weight differences and moisture content, along with grain and groat size parameters, throughout early stages till harvest. Secondly, to analyse differences between top and rest of whorls along the panicle and between primary and secondary grain and groat, for all features above described. Thirdly to investigate the differences between primary and secondary grain and groat at the top and the rest of the panicle by varieties, for all grain traits already mentioned.

Understanding the mechanisms and the physiognomy of grain development may also allow us to improve the uniformity of the grain in terms of physical parameters and at the same time the management conditions of the cultivar. In addition, it might result in a better knowledge of the best time to harvest the grain, to ensure the highest possible values of key quality parameters.

5.2 Materials and Methods

Due to limited information on oat grain development two preliminary experiments were conducted in two different harvest seasons. In 2013/2014, the winter oat varieties Buffalo and Tardis were selected to grow in glasshouse conditions, as a first attempt to establish preliminary characteristics of each grain developmental stage relative wheat and barley (Tottman, 1987; AHDB Cereals & Oilseed, 2009b, 2009a).

Buffalo and Tardis were selected because of their contrasting plant architecture, Buffalo being a dwarf variety and Tardis a conventional height type. In the 2014/2015 harvest season, they were grown under field conditions. For both preliminary experiments, panicles were sampled at a range of stages from flowering time until harvest (early milk, hard dough and ripening), and threshed by hand. The spikelets in each panicle were hard threshed and divided in to primary, secondary, and tertiary grain (when present). The different types of grain were analysed with MARVIN software to obtain the grain area, length and width. After dehulling by hand, MARVIN analysis was repeated to obtain groat area, length and width. The fresh and dry weights (obtained after drying in an oven for 24 h at 60ºC), were measured in order to calculate moisture content.

Both preliminary experiments allowed the establishment of approximate timing of the most suitable collection dates for a more detailed study. At the same time comparisons and differences in grain and groat weight and kernel content through all the main stages of development were also established.

A histogram of frequencies was calculated (data non-shown) for grain and groat area, length and width, of primary, secondary and tertiary (when present). Results suggested a reduction in the range of the data for the three parameters, i.e. grain and groat area, length and width, as grain development progressed. The physiology of the development process and the loss of moisture when the seed is maturing could explain these results.

Based on these experiments, a final field trial was designed in 2015/2016 harvest season with three winter oat varieties, Buffalo, Tardis and Mascani. Mascani was included due to its importance in the market. Field trials were conducted under standard management conditions (see chapter two material and methods). The main stem of individual plants was tagged in the field plots and the physiological changes over time were studied.

From sowing date, the most significant dates of change in developmental stage were obtained from GS39, i.e. flag leaf was fully emerged on the 29th of March 2016, and from this point the first date of sampling was established, milky stage (GS 70), the 25th of June 2016. Further six points of sampling were established in successive dates as shown in table 5.1.

Table 5.1 *Growth stages and dates of sampling from Early milk (GS 70) to Hard dough (GS 86), for Buffalo, Mascani and Tardis. This growth stages were established according to wheat and barley growth guidance* (AHDB Cereals & Oilseed, 2009b, 2009a).

Growth stage	*Dates of sampling*
Early milk (GS70)	25/06/2016
Early milk-Late milk (GS 73-GS75)	28/06/2016
Late Milk (GS77)	09/07/2016
Late milk-Soft dough (GS 82)	14/07/2016
Soft Dough (GS 85)	22/07/2016
Soft dough-Hard dough (GS 83)	25/07/2016
Hard Dough (GS 85)	27/07/2016
Ripening (GS 90)	12/08/2016

To avoid the possible effects previously found on grain and groat size physical traits of removing panicles from the same individual oat plant (data non-shown), the main stem panicle was taken from three different individual plants from each variety in the field at each sampling time. Each panicle was threshed and the panicle divided into 4 portions representing the whorls, labelled from the flag leaf, to the top of the panicle (figure 5.3). The spikelets in each whorl fraction were further split into primary and secondary grain. Grain and groat area, length and width were analysed by image analysis (MARVIN) before and after dehulling by hand. Groat fresh and dry weight and moisture content were determined as described above.

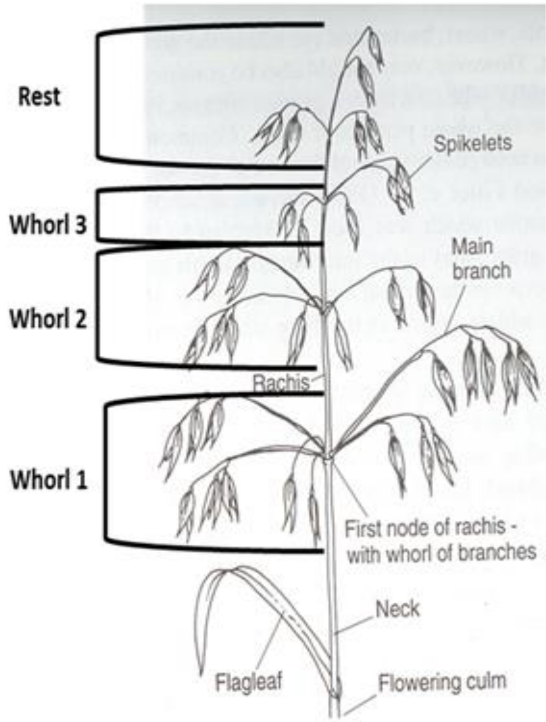


Figure 5.3. *Scheme of sampling of each oat panicle. The characters used as cultivar descriptors are showed*(National Institute of Agricultural Botany (Great Britain), McGarel, & Ardies, 2000).

To establish differences along time and among varieties, statistical analysis, multivariate ANOVA was carried out over the data as follows:

*Treatment: Variable response= (Variety*Type of grain*Growth stage);*

Blocking structure = Position in the panicle (whorl);

Therefore, variety and growth stage were factors and grain and groat area, length, width, kernel content, grain moisture content, and thousand grain weight, variable responses. Comparisons between growth stages, varieties and primary and secondary grain and groat were obtained.

Mean values, by type of grain and position in the panicle, of these physical and grain quality parameters were obtained and plotted. Also, frequency distributions were analysed graphically against growth stage. Reasons beyond this investigation lead to harvest before sampling at ripening, and therefore losing that final stage of grain development in the statistical analysis and graphical representation.

5.3 Results

5.3.1. Kernel content

Kernel content showed significant differences between grain development stages (figure 5.4), with higher values at late milk/soft dough. Final mean values at hard dough showed no differences with early stages mean values.

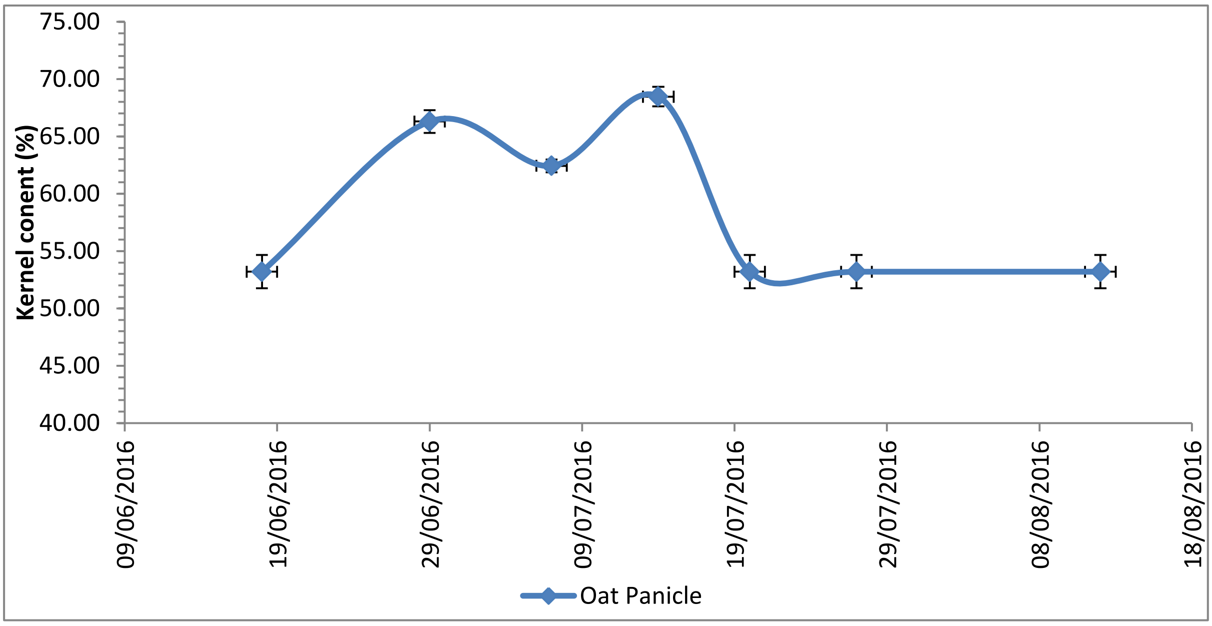


Figure 5.4 *Mean grain kernel content (%) ± s.e.m. of Buffalo, Mascani and Tardis panicle from early milk (18/06/2016) to hard dough (12/08/2016).*

Secondly, the panicle was analysed at the top of the panicle, dividing at the same time between primary and secondary grain (figure 5.5). Interestingly, secondary grain had greater levels of kernel content in comparison with primary grain through all grain development stages, reaching higher mean values at soft dough-hard dough (27/07/2016) (figure 5.5).

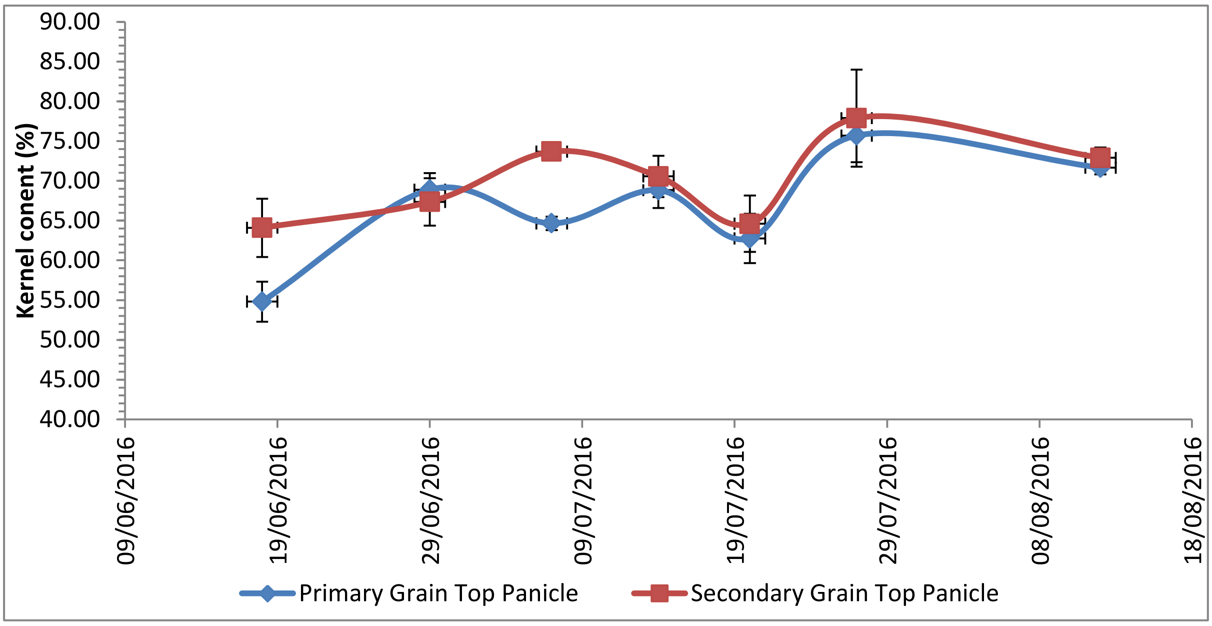


***Figure 5.5** Mean grain kernel content (%) ± s.e.m. by primary and secondary grain at the top of the panicle of Buffalo, Mascani and Tardis, from early milk to hard dough.*

There were non-significant differences (p-value>0.05) within primary and secondary grain when comparing within variety at any growth stage. However there were significant differences (p-value<0.001) between primary and secondary grain kernel content between the top and rest of the panicle and between varieties. Similar patterns of development in kernel content of Buffalo and Tardis (figure 5.6.a and 5.6.b and table 5.2) were found in the top and rest of the panicle and in primary and secondary grain. Both varieties showed lower values at early milk (GS 70) increasing throughout development. Mascani displayed a more irregular pattern (table 5.2) with higher values at early milk-late milk (29/06/2016) in comparison with other growth stages. In contrast to Buffalo and Tardis, kernel contents were higher for Mascani primary grain than for secondary grain at the final sampling point (figure 5.6.a and 5.6.b and table 5.2).

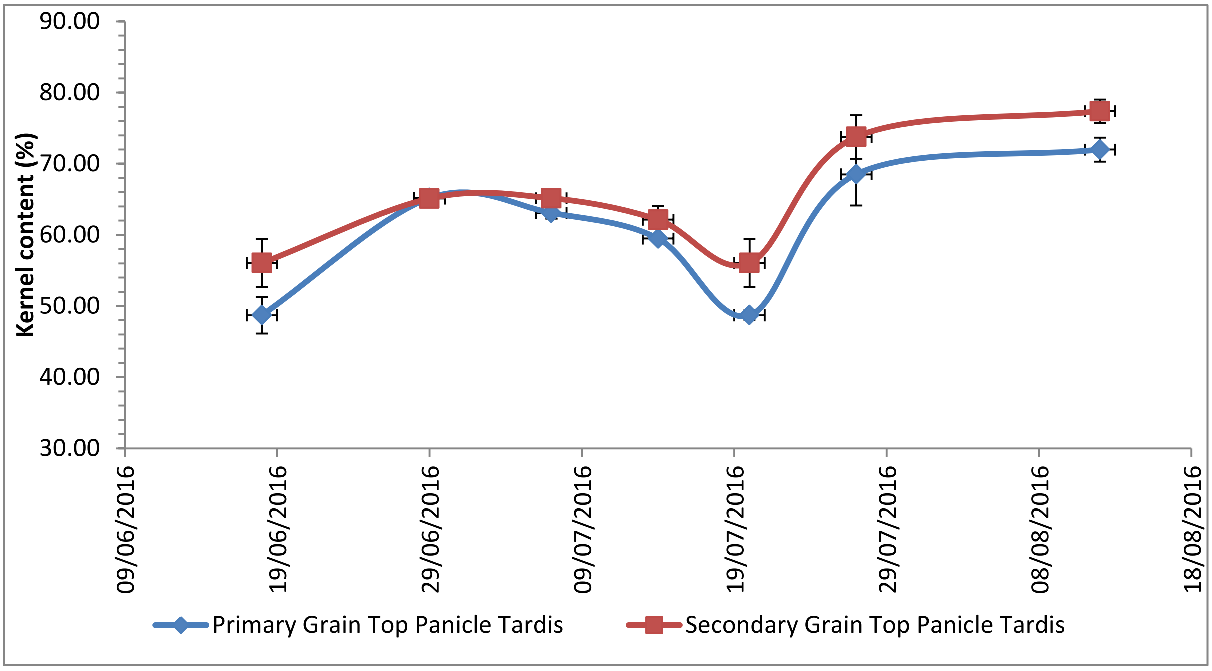


Figure 5.6.a *Mean grain kernel content (%) ± s.e.m at the top of Tardis panicle and by primary and secondary grain from early milk to hard dough.*

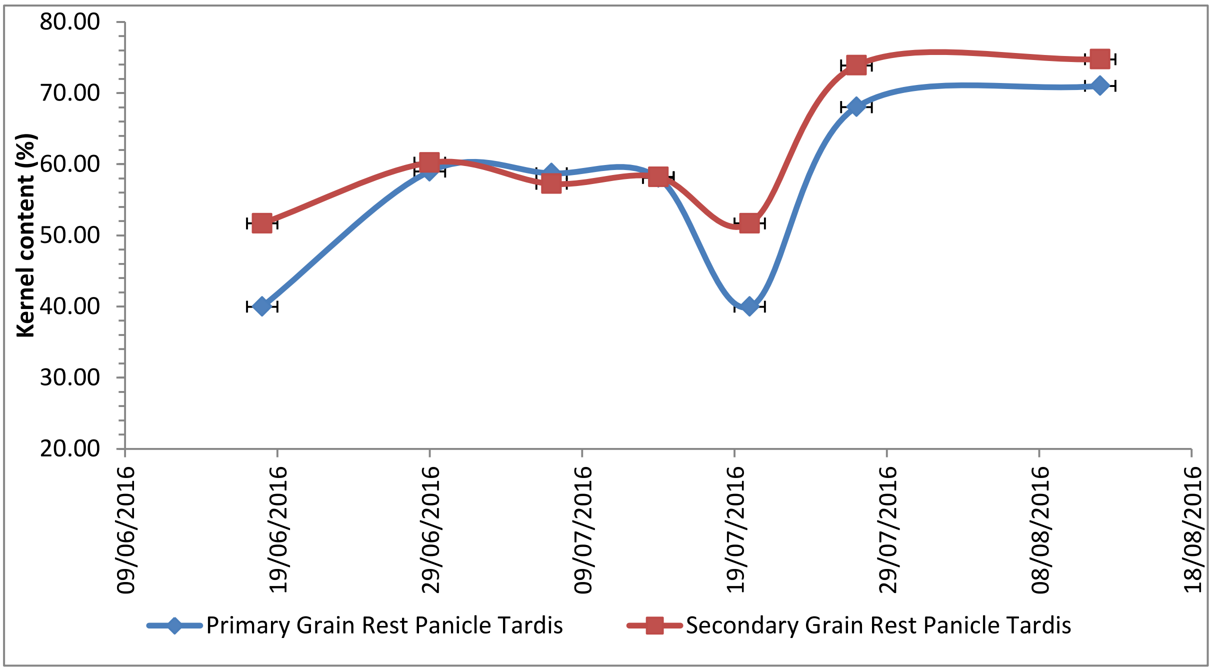


Figure 5.6.b *Mean grain kernel content (%) ± s.e.m in the rest of Tardis panicle and by primary and secondary grain from early milk to hard dough.*

Table 5.2 *Mean grain kernel content (%) ± s.e.m. at the top and rest of Buffalo and Mascani panicle by primary and secondary grain.*

	Top of the panicle				Rest of the panicle			
	Prim		Sec		Prim		Sec	
Buffalo	Kernel Content	s.e.m.	Kernel Content	s.e.m.	Kernel Content	s.e.m.	Kernel Content	s.e.m.
Early milk	50.46	1.934	56.76	3.158	39.39	2.484	42.16	3.338
Early milk Late milk	63.48	1.917	67.89	3.159	55.03	1.232	63.58	1.405
Late milk	63.27	1.552	70.19	0.684	55.48	1.141	64.60	1.175
Late milk Soft dough	69.74	0.827	76.55	0.865	62.04	0.957	72.80	1.311
Soft dough	71.45	0.818	83.26	0.529	71.05	1.368	81.67	3.469
Soft dough Hard dough	71.90	6.249	69.70	5.970	72.08	2.851	65.35	3.160
Hard dough	71.17	1.679	79.70	2.372	66.96	2.559	79.60	2.217
	Prim		Sec		Prim		Sec	
Mascani	Kernel Content	s.e.m.	Kernel Content	s.e.m.	Kernel Content	s.e.m.	Kernel Content	s.e.m.
Early milk	65.27	2.567	79.51	2.741	60.31	2.049	73.85	3.037
Early milk Late milk	78.04	0.873	85.67	1.995	71.93	2.740	81.12	1.601
Late milk	67.63	0.807	66.74	2.179	65.18	1.117	68.01	0.850
Late milk Soft dough	77.27	0.451	82.35	0.561	74.84	0.587	80.64	0.544
Soft dough	68.10	0.702	72.40	0.910	66.76	0.627	69.01	1.314
Soft dough Hard dough	86.67	4.382	50.35	16.217	79.78	1.897	58.62	5.556
Hard dough	71.78	1.688	76.58	2.895	70.82	1.260	76.16	1.721

5.3.2. Thousand Grain and Groat Weight

Thousand grain weight differed significantly between growth development stages (figure 5.7) (p-value<0.001, MANOVA), maximizing between late milk (7/07/2016) and soft dough (14/07/2016) and reaching minimum values at soft dough with little variation until hard dough and showing similar mean values when compared to early milk (figure 5.7).

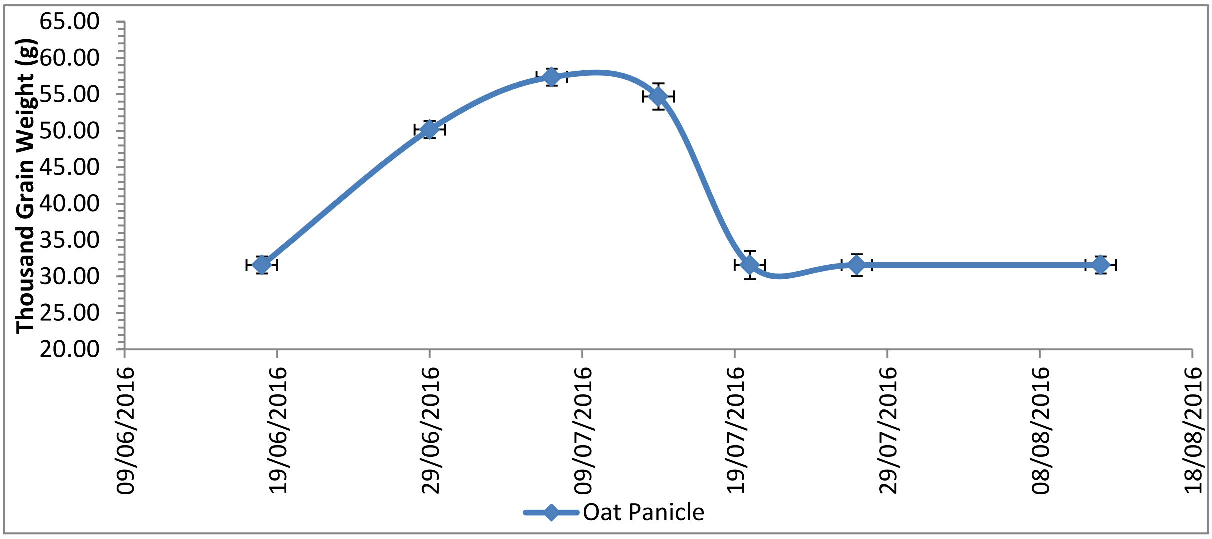


***Figure 5.7** Mean thousand grain weight values (g) ± s.e.m. along growth development stages from early milk to hard dough of Buffalo, Mascani and Tardis.*

Variety and growth stage, and type of grain and growth stage interactions were significant (p-value<0.05) but interactions between type of grain and variety were non-significant as were the effects of variety, type of grain and growth stage (p-value>0.05).

There were significant differences (p-value<0.001) between primary and secondary grain kernel content top and rest of the panicle (figure 5.8.a. and 5.8.b). At the top of the panicle primary and secondary grain thousand grain weight was always higher than the rest of the panicle (figure 5.8.a and 5.8.b and table 5.3). Higher mean thousand grain weight values were found at the end of growth development (figure 5.8.a and 5.8.b and table 5.3).

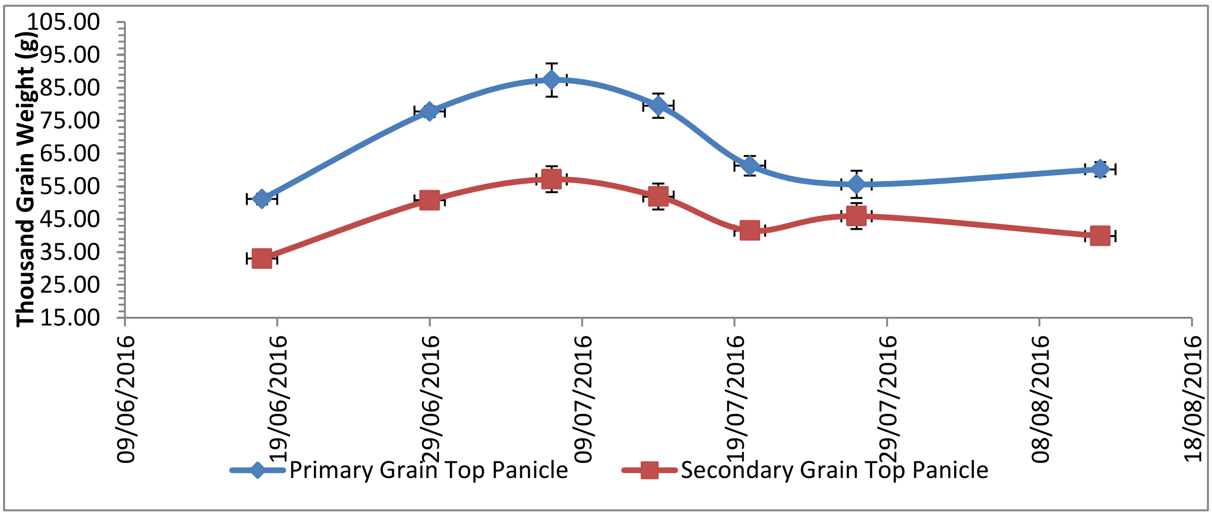


***Figure 5.8.a** Mean thousand grain weight values (g) ± s.e.m at the top of Buffalo, Mascani and Tardis panicle and by primary and secondary grain from early milk to hard dough.*

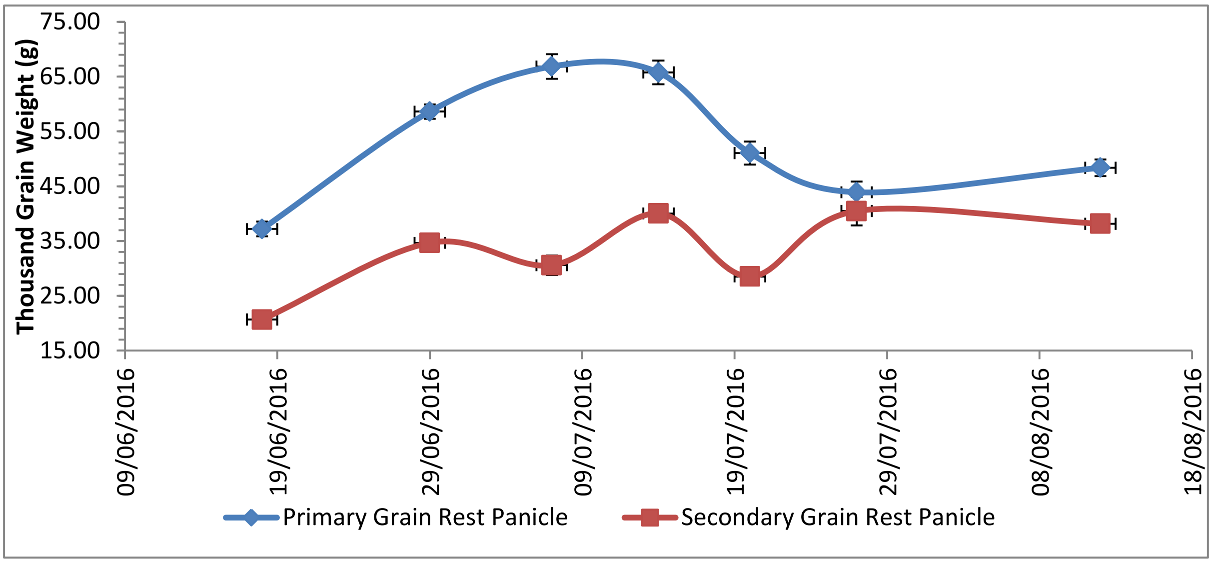


Figure 5.8.b *Mean thousand grain weight values (%) ± s.e.m at the rest Buffalo, Mascani and Tardis panicle and by primary and secondary grain from early milk to hard dough*

All varieties had higher primary grain thousand grain weight at all stages of grain development, in comparison with secondary grain (figure 5.9 and table 5.3). However, similar thousand grain weight pattern was found in primary grain in the lower whorls and secondary grain in the top whorls.

Tardis thousand grain weight mean values from primary and secondary grain had similar growth development (figure 5.9.a and 5.9.b). Both primary and secondary grain by whorls, had maximum values at soft dough (22/07/2016), decreasing slightly until hard dough (12/08/2016).However, Tardis displayed two different patterns of development when the thousand grain weight mean values were analysed by whorls (figure 5.9.a and 5.9.b). The top of the panicle, reached highest mean thousand grain weight at late milk slightly decreased until hard dough. The rest of the panicle reached highest thousand grain weight at soft dough (figure 5.9.a. and 5.9.b), decreasing abruptly at hard dough to end with similar values, when compared to the top of the panicle.

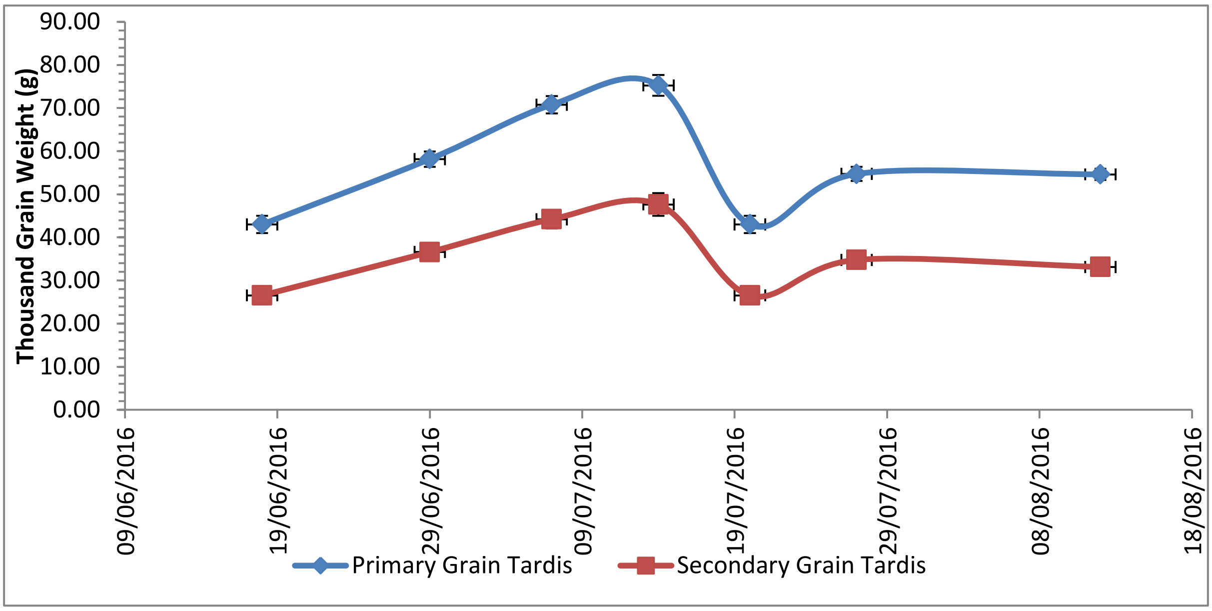


Figure 5.9.a *Mean thousand grain weight values (g) ± s.e.m of Tardis top panicle and by primary and secondary grain from early milk to hard dough.*

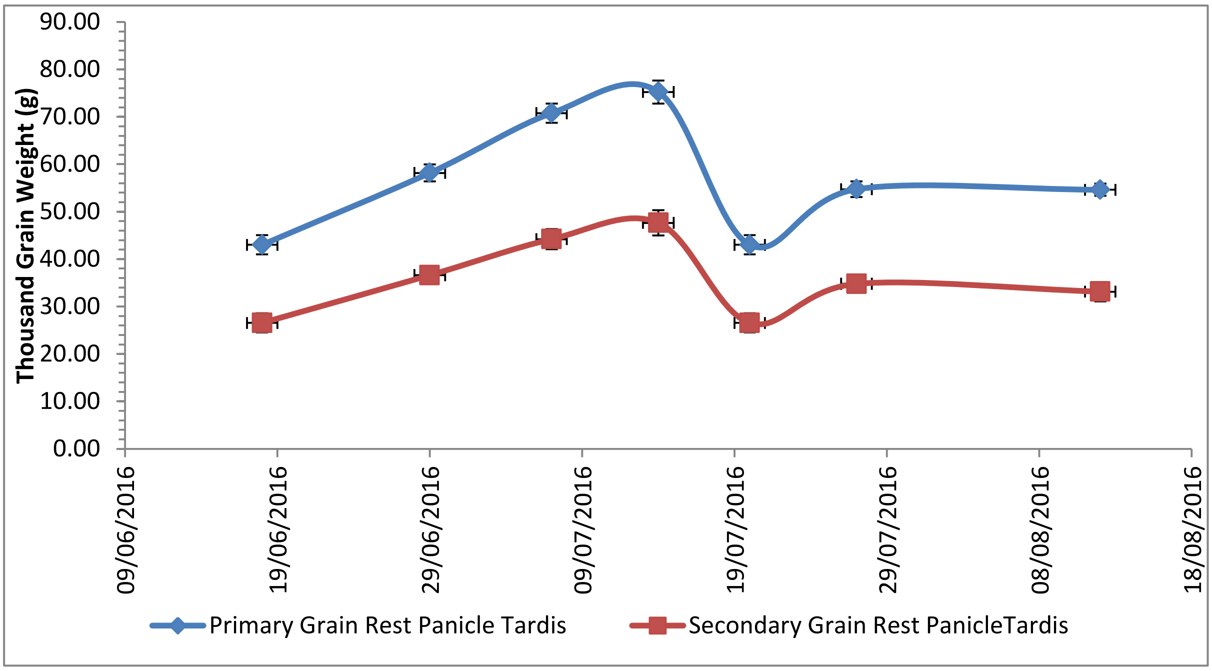


Figure 5.9.b *Mean thousand grain weight values (g) ± s.e.m in of Tardis rest of panicle and by primary and secondary grain from early milk to hard dough.*

Table 5.3 *Mean thousand grain weight (g) values ± s.e.m. at the top and rest of Buffalo and Mascani panicle.*

	Top of the panicle				Rest of the panicle			
	Prim		Sec		Prim		Sec	
Buffalo	Thousand Grain Weight	s.e.m.	Thousand Grain Weight	s.e.m.	Thousand Grain Weight	s.e.m.	Thousand Grain Weight	s.e.m.
Early milk	51.23	1.162	28.90	1.374	37.07	1.856	17.83	1.084
Early milk Late milk	74.67	2.559	44.59	2.783	54.59	1.937	30.91	1.245
Late milk	70.05	5.708	40.42	3.342	52.67	2.238	29.73	1.425
Late milk Soft dough	64.78	4.526	36.84	3.251	49.63	2.239	27.07	1.136
Soft dough	52.54	3.420	34.33	2.742	42.59	2.799	26.25	1.605
Soft dough Hard dough	49.70	10.672	33.75	8.510	33.62	2.610	25.43	2.190
Hard dough	54.20	1.633	30.99	2.560	38.51	1.999	20.92	1.541
	Prim		Sec		Prim		Sec	
Mascani	Thousand Grain Weight	s.e.m.	Thousand Grain Weight	s.e.m.	Thousand Grain Weight	s.e.m.	Thousand Grain Weight	s.e.m.
Early milk	44.94	0.303	29.32	1.206	31.51	2.235	17.69	1.738
Early milk Late milk	82.41	1.403	54.44	1.678	63.10	2.585	36.51	2.234
Late milk	105.19	5.641	66.07	3.575	77.09	4.257	47.50	3.394
Late milk Soft dough	85.28	2.933	53.18	2.335	72.39	2.558	39.75	1.743
Soft dough	74.05	1.224	49.38	1.862	67.60	1.669	38.98	2.075
Soft dough Hard dough	50.36	1.962	53.55	3.989	43.38	3.331	59.88	3.746
Hard dough	61.57	5.124	40.49	4.672	52.01	2.528	31.56	2.032

Buffalo thousand grain weight of primary and secondary grain showed similar development (table 5.3) when compared to the overall mean thousand grain weight values by panicle sections. At the top of the panicle primary and secondary grain thousand grain weight was always higher than to the rest of the panicle. In both cases, lower mean thousand grain weights were found at the end of grain development.

Mascani had higher thousand grain weight at Late milk (GS77) (9/07/2016) (table 5.3) at the top of the panicle whilst the rest of the panicle reached higher thousand grain weight at soft dough (22/07/2016). The whole panicle had smaller thousand grain weight at the end of growth development.

5.3.3. Moisture content

Moisture content differed significantly between grain development stages (figure 5.10) (p-value<0.001, MANOVA), maximizing between early milk/late milk (7/07/2016) and reaching minimum values at soft dough with little variation until hard dough and showing similar mean values when compared to early milk (figure 5.10).

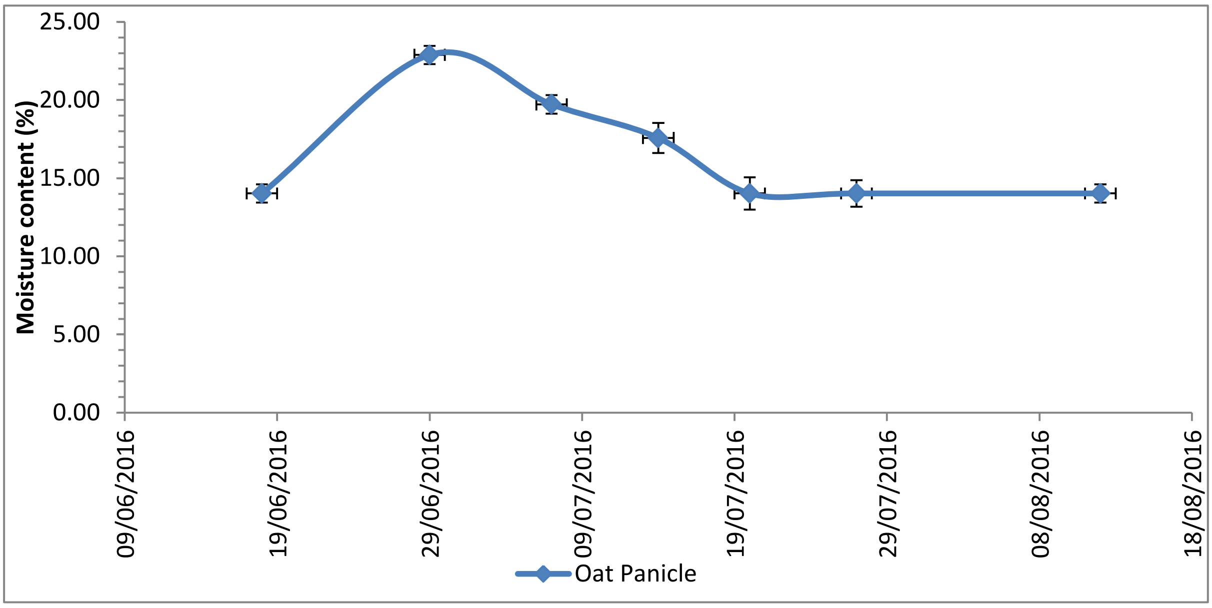


Figure 5.10 *Mean moisture content (%) ± s.e.m. along grain development stages from early milk to hard dough of Buffalo, Mascani and Tardis.*

There were significant differences (p-value<0.001) between primary and secondary grain kernel content top and rest of the panicle (figure 5.11.a. and 5.11.b). At the top of the panicle primary and secondary grain thousand grain weight was higher than the rest of the panicle (figure 5.11.a and 5.11.b). However, they had similar moisture content pattern through grain development.

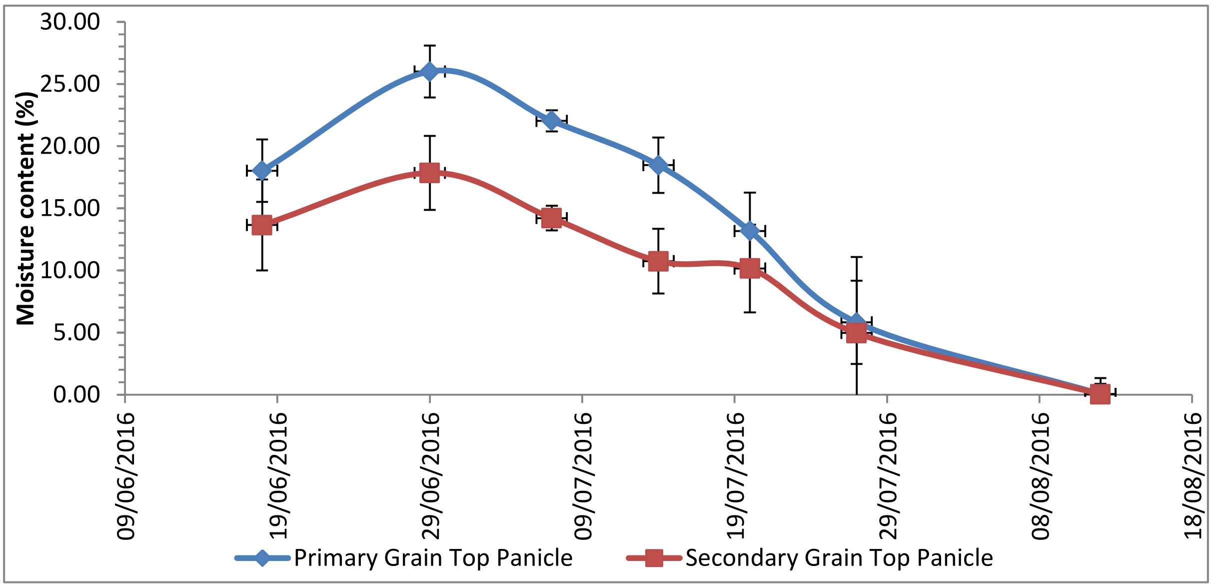


Figure 5.11.a *Mean moisture content (%) ± s.e.m of Buffalo, Mascani and Tardis top panicle and by primary and secondary grain from early milk to hard dough.*

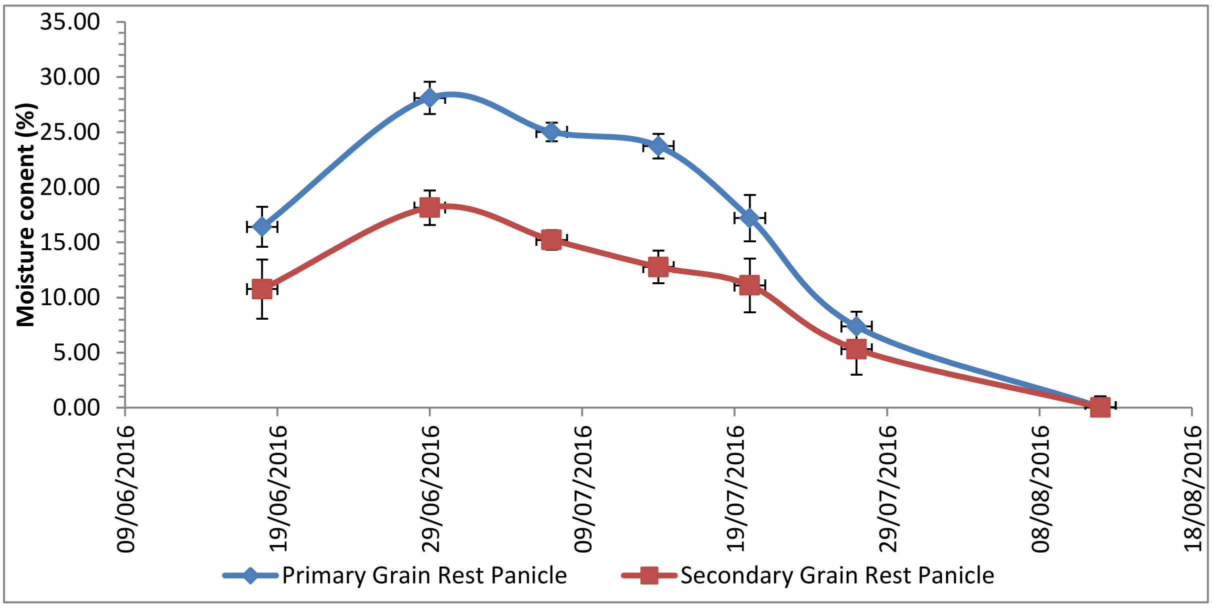


Figure 5.11.b *Mean moisture content (%) ± s.e.m of Buffalo, Mascani and Tardis top panicle and by primary and secondary grain from early milk to hard dough.*

For all varieties and for both primary and secondary grain, moisture content was significantly different (p-value<0.001, MANOVA) between growth stages and varieties, but no significant interactions were found between any factor (p-value>0.05).

All varieties showed higher percentages of moisture contents when analysed at the top and rest of the panicle at the beginning of the development (table 5.4), i.e. early milk

(GS 70). The top of the panicle of the three varieties had lower moisture contents at the beginning of grain development but higher at the end, when compared to lower whorls.

***Table 5.4** Mean moisture content (%) by whorls, of Buffalo, Mascani and Tardis, along grain development stages, from early milk (GS 70) to ripening (GS90), by primary and secondary grain.*

		Whorl 1		***Whorl 2***		***Whorl 3***		***Top***	
Varieties		***Primary***	***Secondary***	***Primary***	***Secondary***	***Primary***	***Secondary***	***Primary***	***Secondary***
Mascani	Early Milk	61%	63%	64%	63%	60%	63%	58%	60%
	Late Milk	50%	52%	46%	52%	49%	51%	46%	45%
	Soft Dough	53%	48%	51%	46%	50%	45%	47%	50%
	Hard Dough	37%	37%	36%	34%	34%	33%	27%	27%
Tardis	Early Milk	58%	59%	59%	60%	57%	57%	54%	57%
	Late Milk	48%	49%	46%	47%	45%	45%	43%	41%
	Soft Dough	50%	48%	47%	45%	37%	37%	33%	32%
	Hard Dough	36%	38%	32%	32%	31%	31%	24%	24%
Buffalo	Early Milk	60%	62%	61%	62%	60%	63%	60%	71%
	Late Milk	51%	51%	50%	50%	47%	48%	44%	44%
	Soft Dough	39%	36%	38%	33%	34%	33%	27%	27%
	Hard Dough	38%	35%	37%	26%	33%	27%	21%	20%

Primary and secondary groats showed different moisture contents during grain development. Thus, in Buffalo (figure 5.12), Mascani (figure 5.13) and Tardis (figure 5.14), both primary and secondary grain, had at mid-stages of grain development, i.e. late milk, soft dough and hard dough, high moisture contents, decreasing at the final growth stage. However, at final growth stages, Buffalo (figure 5.13) and Tardis (figure 5.15) had primary grain moisture contents below 10% whilst Mascani (figure 5.14) primary grain was above 10% moisture content.

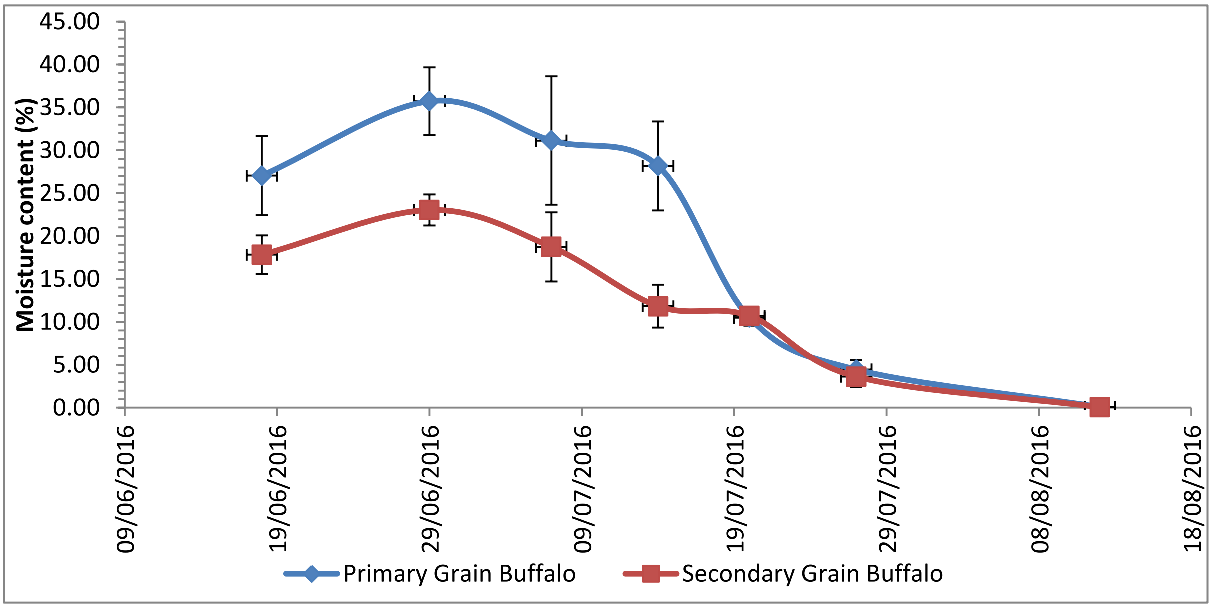


Figure 5.12 *Mean moisture content (%) ± s.e.m. by primary and secondary grain along grain development stages of Buffalo.*

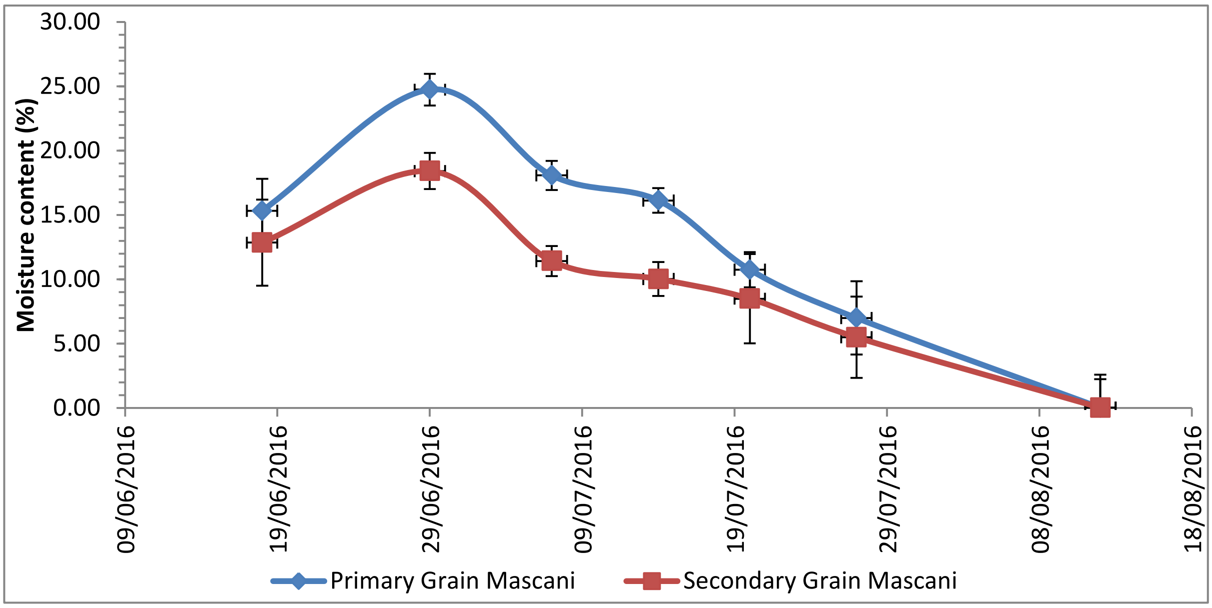


Figure 5.13 *Mean moisture content (%) ± s.e.m. by primary and secondary grain along grain development stages of Mascani.*

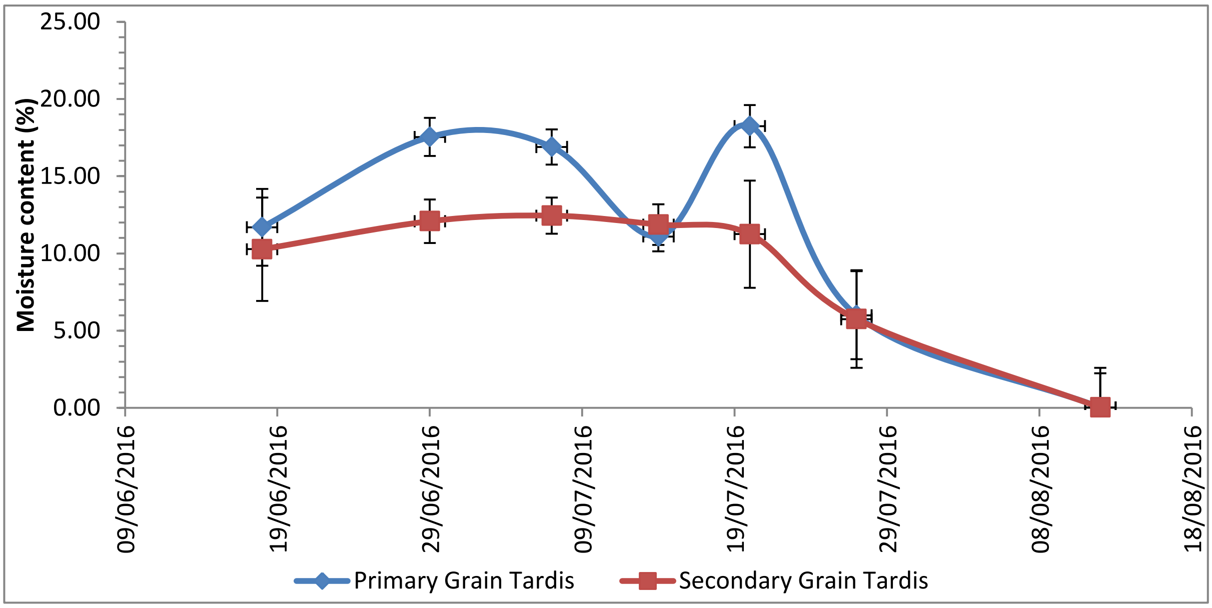


Figure 5.14 *Mean moisture content (%) ± s.e.m. by primary and secondary grain along grain development stages of Tardis.*

5.3.4. Grain and groat size and shape

5.3.4.1 Grain Area

Mean grain areas (mm^2) were significantly different (p-value<0.001, MANOVA) for growth stages (figure 5.15). The general pattern showed maximum grain areas between soft dough and hard dough (figure 5.15) decreasing at the end reaching similar values to early stages.

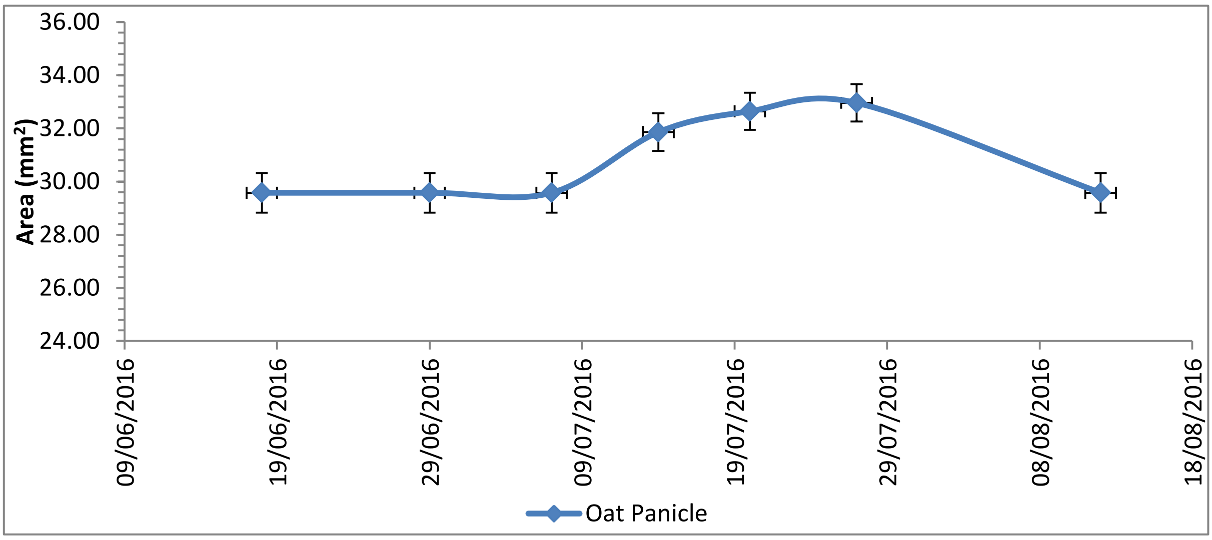


Figure 5.15 *Mean grain area (mm^2) ± s.e.m. by primary and secondary grain along grain development stages of Buffalo, Mascani and Tardis.*

A similar pattern was found when dividing the panicle between the top and the rest of whorls analysing grain areas, although higher grain areas were found at the top (figure 5.16.a. and 5.16.b)

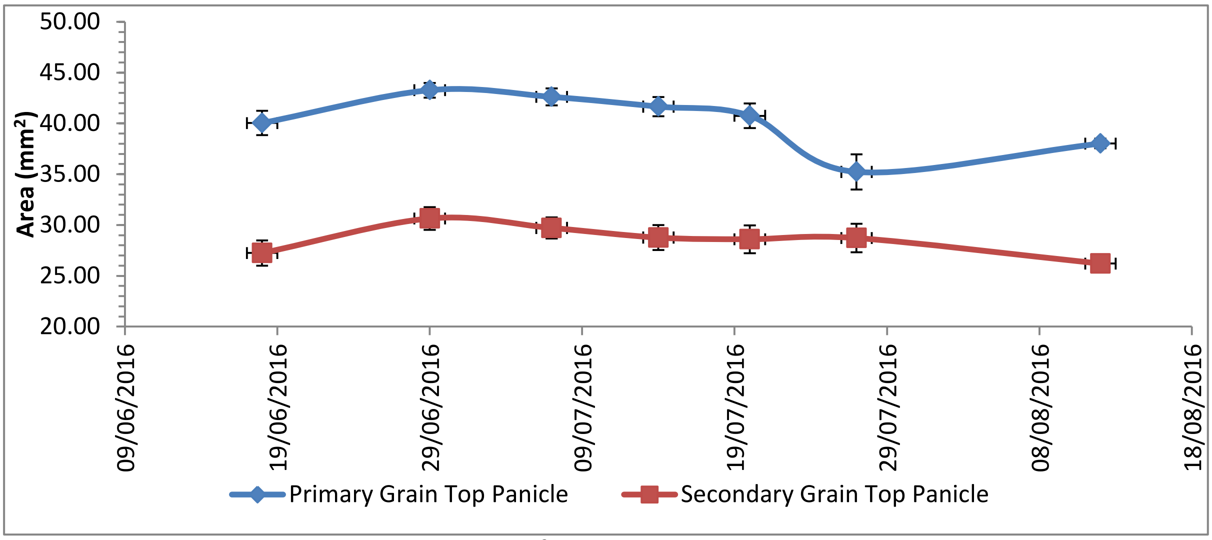


Figure 5.16.a *Mean grain area (mm^2) ± s.e.m. by primary and secondary grain along grain development stages of Buffalo, Mascani and Tardis top panicle.*

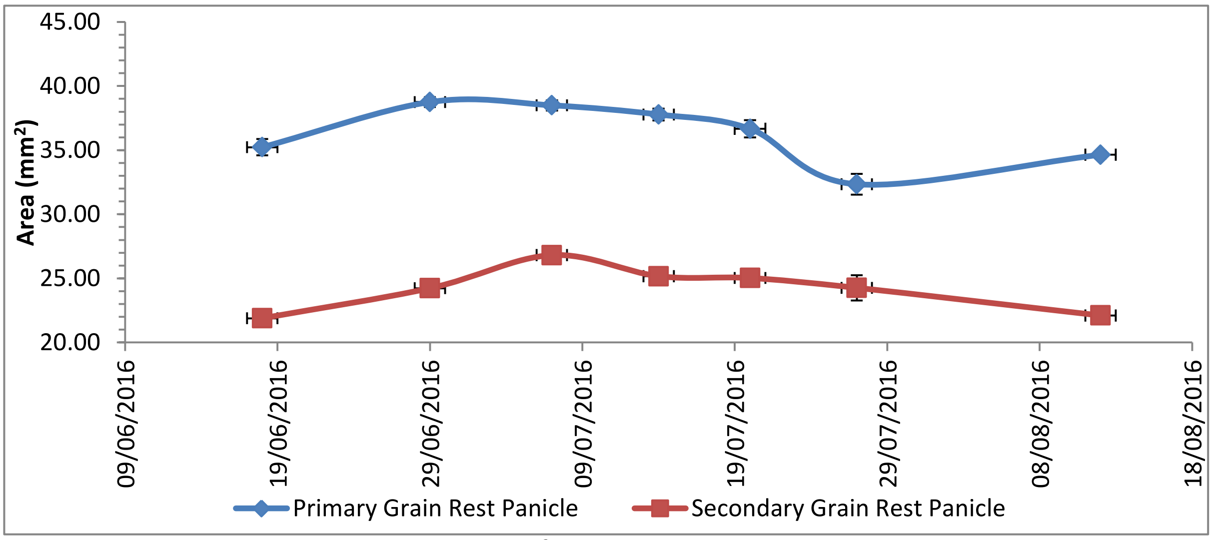


Figure 5.16.b *Mean grain area (mm^2) ± s.e.m. by primary and secondary grain along grain development stages of Buffalo, Mascani and Tardis, rest of the panicle.*

Significant differences were also found in grain area between varieties, type of grain, variety and growth stage and interactions between variety, type of grain and growth stage (p-value<0.001).

Buffalo mean grain areas (mm^2) (figure 5.17) were higher between early milk and late milk and lower at the final growth stage at both top and rest of the panicle showing similar pattern of development. However, mean grain area at the top of the panicle displayed higher values when compared to lower whorls. There were no differences between in grain area at early milk and final stages of grain development.

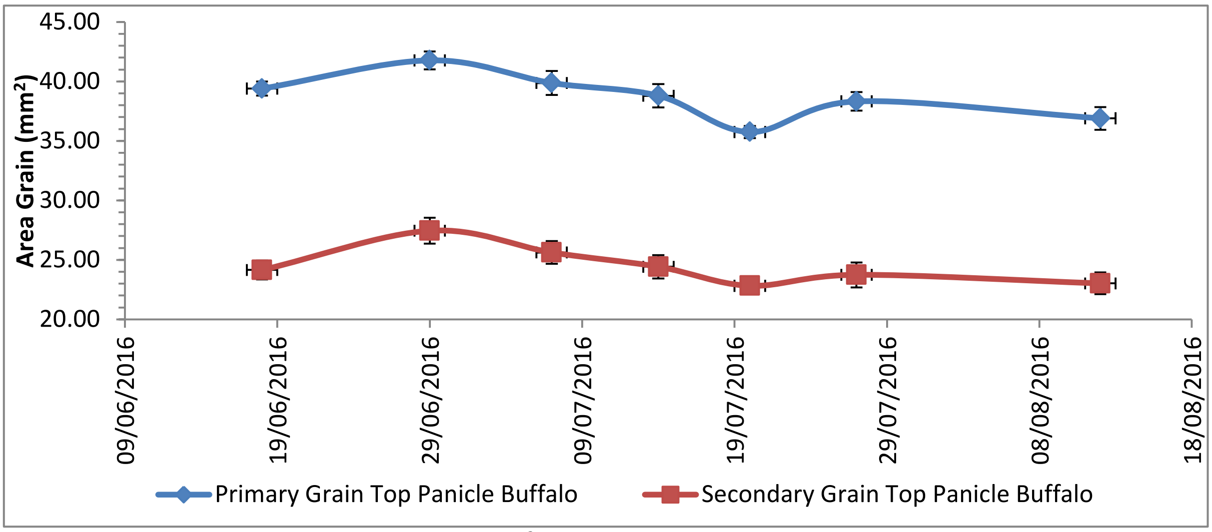


Figure 5.17 *Mean grain area (mm^2) ± s.e.m. top panicle values by primary and secondary grain throughout grain development stages of Buffalo.*

Mascani mean grain areas (mm^2) were highest at the top of the panicle (figure 5.18), similar to Buffalo and Tardis. However the time pattern was different to that of Buffalo and Tardis. Thus, higher for Mascani grain area were found at late milk (figure 5.18). Interestingly, final mean grain areas were similar to values found at the beginning of grain development. Mascani top and rest of the panicle showed the same pattern in grain area development.

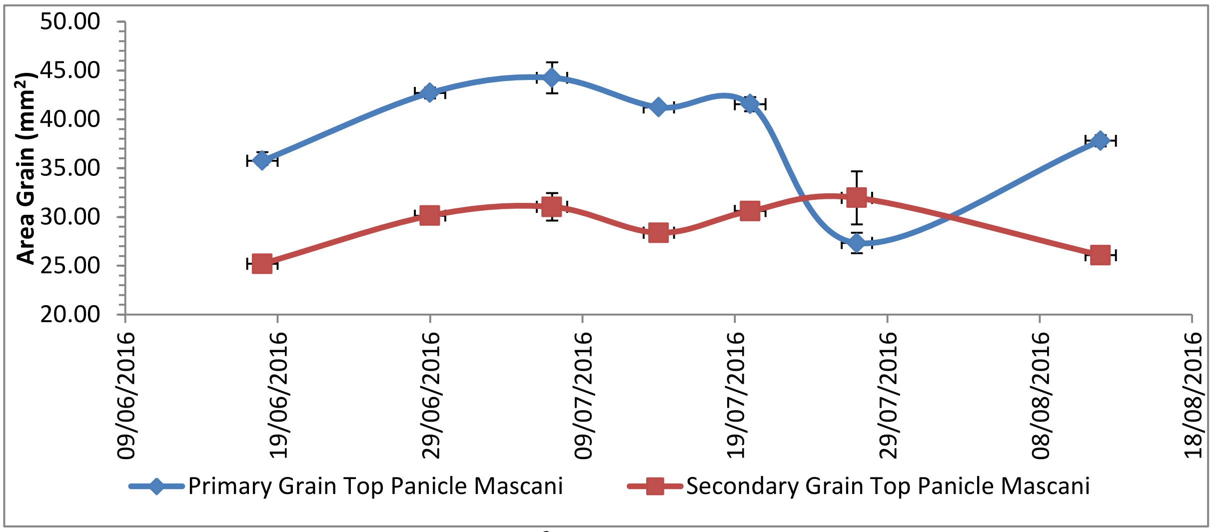


Figure 5.18 *Mean grain area (mm^2) ± s.e.m. throughout grain development stages of Mascani top panicle, by primary and secondary grain.*

Tardis mean grain areas (mm^2) were higher at the top of the panicle (figure 5.19), similar to Buffalo and Mascani and with no differences in time pattern between the top and the rest of panicle. Higher Tardis grain areas were found between early milk and late milk and decreased at the end of grain development (figure 5.18). Interestingly, final mean grain areas were lower than values found at the beginning of grain development.

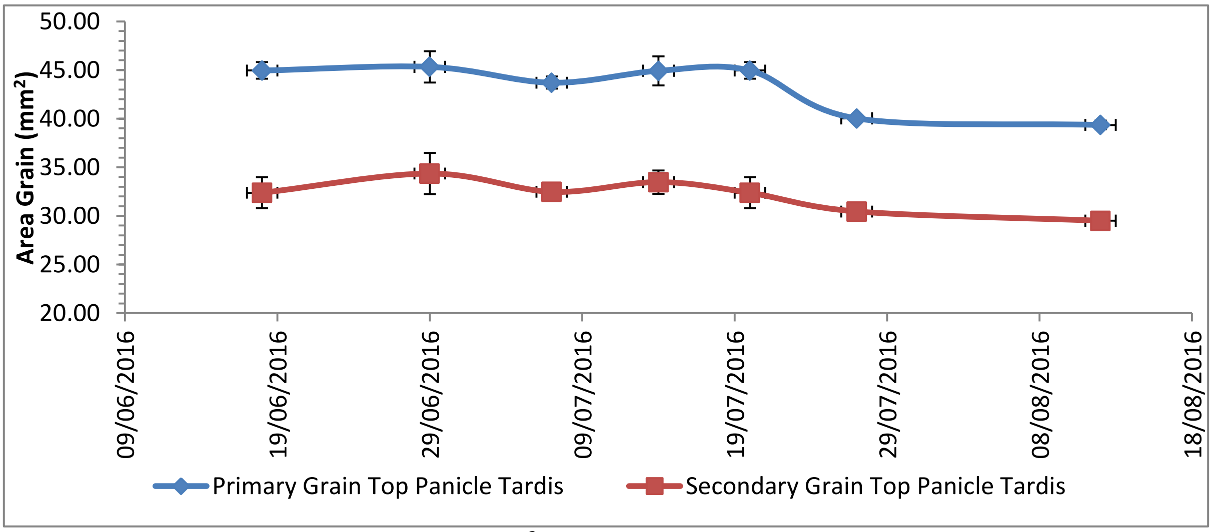


Figure 5.19 *Mean grain area (mm^2) ± s.e.m. throughout grain development of Tardis top panicle, by primary and secondary grain.*

Rate of change of the primary and secondary grain mean grain areas (table 5.5) were calculated as a rate of change for each type of grain, i.e. primary and secondary, and at each growth stage in comparison with the previous growth stage. If this value was negative mean value had decreased compared to the previous growth stages. Time in days between growth stages was also annotated.

Table 5.5 *Rate of change of mean grain area (mm^2) values and days between growth development stages of each variety primary and secondary grain.*

Variety			*Whorl 1*		*Whorl 2*		*Whorl 3*		*Top*	
		Days	*Primary*	*Secondary*	*Primary*	*Secondary*	*Primary*	*Secondary*	*Primary*	*Secondary*
Buffalo	*EM/LM*	10	4%	16%	2%	14%	1%	12%	1%	9%
	LM/SD	12	16%	23%	15%	21%	15%	20%	5%	9%
	SD/HD	7	-1%	-7%	-4%	-7%	-5%	-4%	-3%	-4%
	HD	14	-14%	-11%	-10%	-9%	-10%	-13%	-6%	-8%
Mascani	*EM/LM*	14	-6%	6%	-1%	5%	-4%	-1%	-7%	-5%
	LM/SD	13	10%	9%	4%	5%	7%	8%	0%	8%
	SD/HD	5	3%	4%	4%	5%	6%	8%	5%	2%
	HD	16	-15%	-17%	-12%	-14%	-16%	-19%	-13%	-17%
Tardis	*EM/LM*	12	11%	11%	7%	16%	3%	2%	0%	-2%
	LM/SD	13	8%	8%	3%	0%	1%	6%	0%	0%
	SD/HD	5	-5%	-5%	2%	7%	0%	2%	4%	3%
	HD	16	-3%	-3%	-10%	-11%	-10%	-14%	-13%	-13%

Primary and secondary grain mean grain areas showed significant differences between growth stages (p-value<0.001). The highest rates of growth (table 5.5) were found in the final stages of grain development when they were negative for all varieties. For example, Buffalo, whorl 1 primary grain area showed a -14% decrease at hard dough in comparison with soft dough/hard dough, and Mascani for whorl 3 secondary grain a -19% decrease in grain area at soft dough when compared to hard dough. The smallest growth rates at early stages of development were found at the top of the panicle for both primary and secondary grain area. This was because the grain areas at the top of the panicle were significantly larger at the early milk stage.

5.3.4.2 Grain length

Mean grain length (mm) showed statistically significant differences (p-value<0.001) between varieties, type of grain and growth stages. Significant interactions were found between variety and type of grain, variety and growth stage, between type of grain and growth stage and between variety, type of grain and growth stage (p-value<0.001).

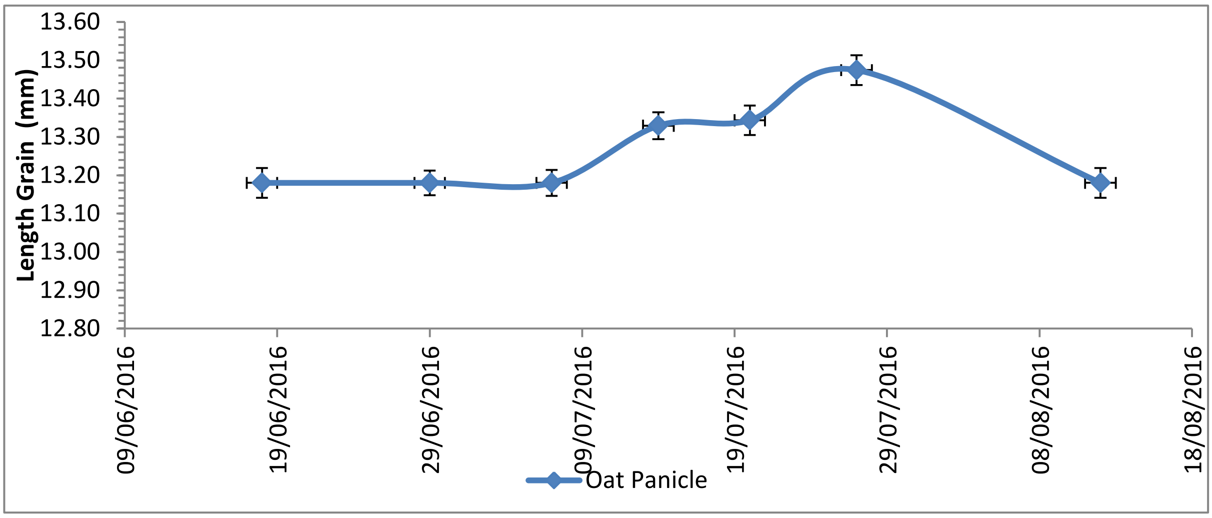


Figure 5.20 *Mean grain length (mm) ± s.e.m. by primary and secondary grain along grain development stages of Buffalo, Mascani and Tardis.*

A similar pattern was found when comparing the top and the rest of the panicle grain length. Higher values were found in both cases at soft dough (figure 5.21.a and 5.21.b), decreasing at the final stages reaching similar values compared to early stages.

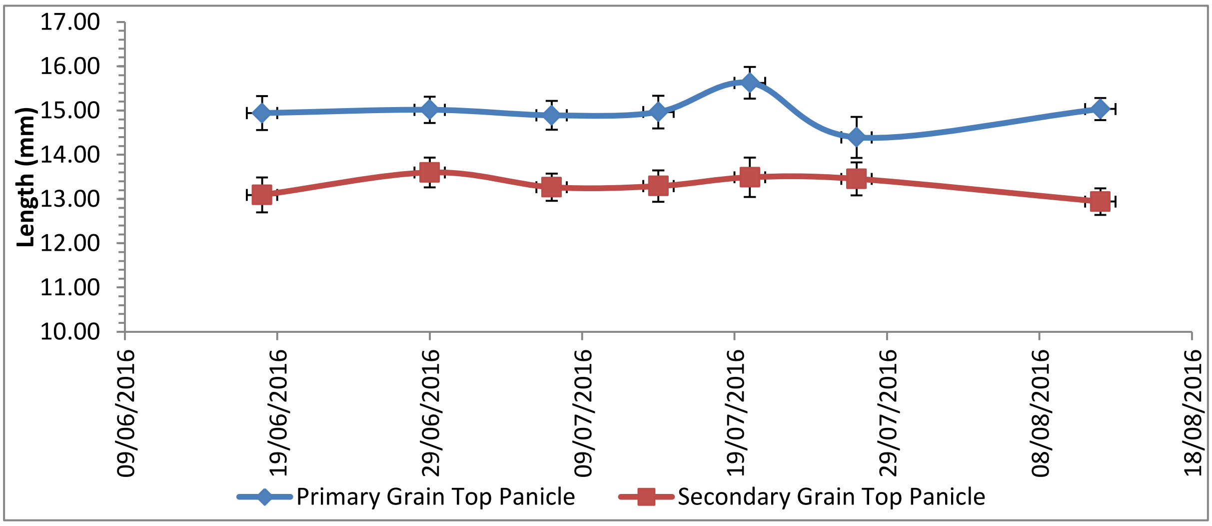


Figure 5.21.a *Mean grain length (mm) ± s.e.m. by primary and secondary grain along grain development stages of Buffalo, Mascani and Tardis, top panicle.*

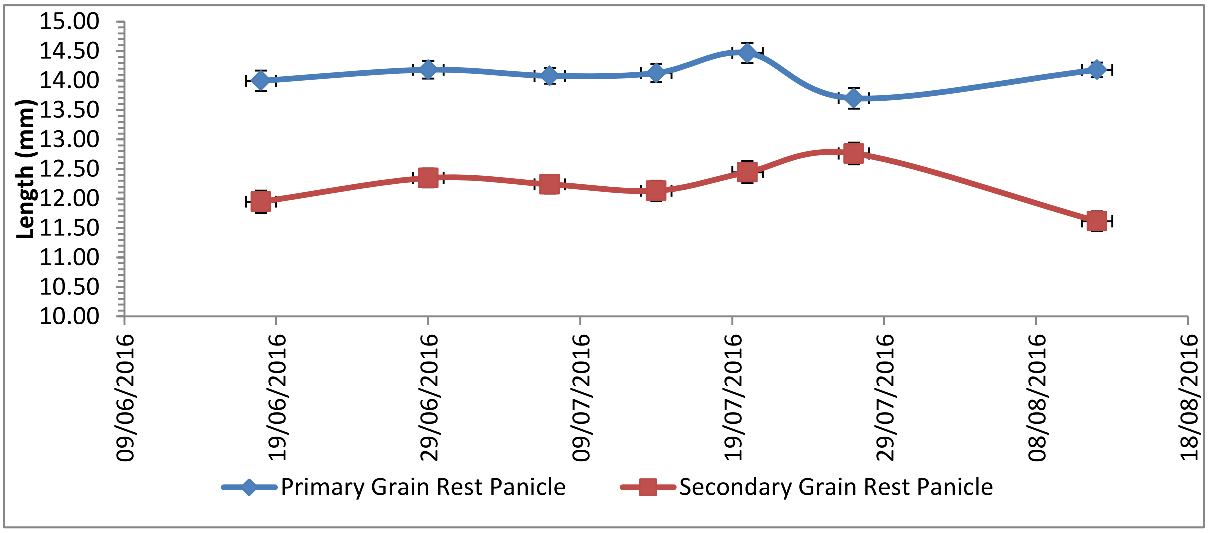


Figure 5.21.b *Mean grain length (mm) ± s.e.m. by primary and secondary grain along grain development stages of Buffalo, Mascani and Tardis, rest of whorls panicle.*

Buffalo mean grain length was slightly greater at the end of grain development (12/08/2016) and top panicle whilst the rest of whorls were longer at soft dough (22/07/2016) (figure 5.22.a and 5.22.b). The time pattern observed for both top and rest of the panicle were similar.

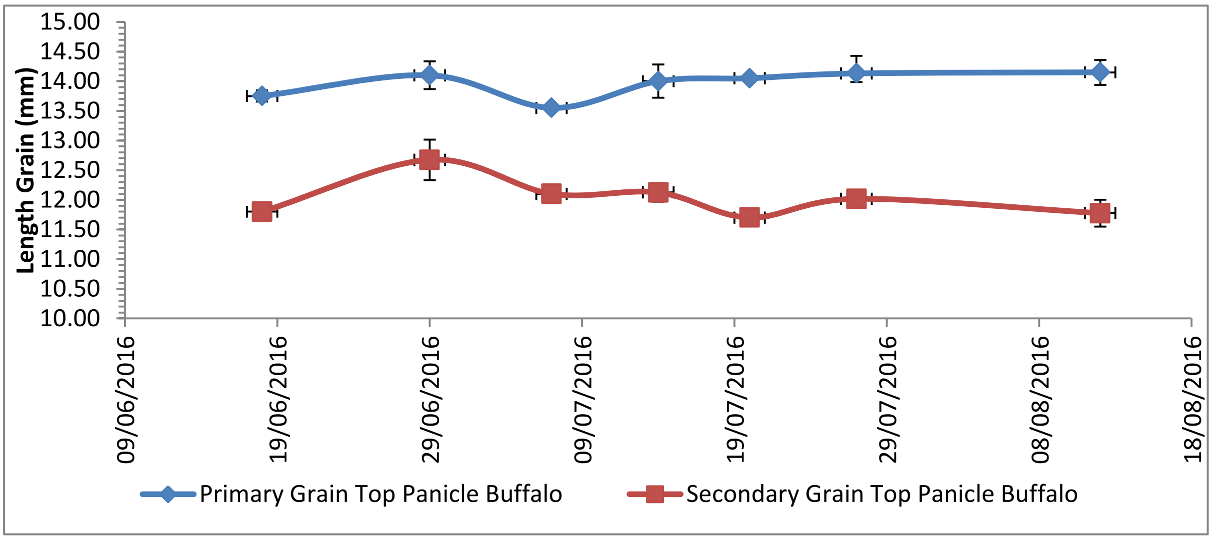


Figure 5.22.a.*Mean grain length (mm) ± s.e.m. throughout grain development of Buffalo top panicle by primary and secondary grain.*

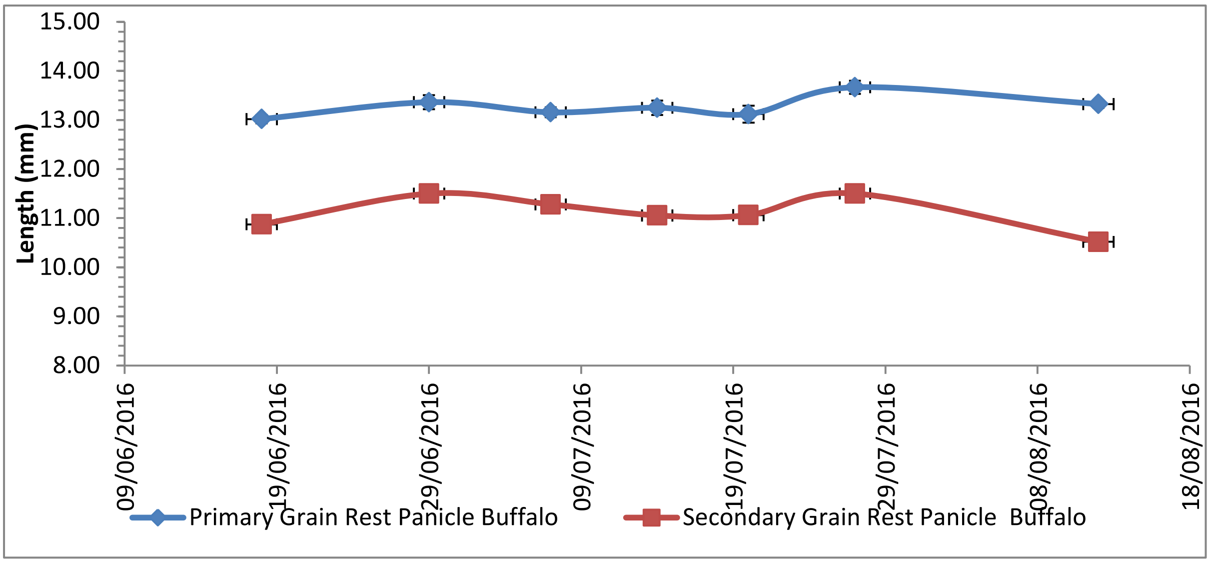


***Figure 5.22.b** Mean grain length (mm) ± s.e.m. throughout grain development of Buffalo rest of the panicle by primary and secondary grain.*

Mascani top panicle mean grain length (mm) (figure 5.23.a) was higher at soft dough (20/07/2016) and decreased at the end of grain development to similar values to early milk (25/07/2016). There was an odd grain primary length time pattern between soft dough and hard dough (figure 5.23.a), with secondary grain reaching higher grain length than primary grain. Although this is could be due to an odd replicate in the sampling process rather than a characteristic grain length development, the same pattern was found when analysing the rest of the panicle grain length (figure 5.23.b).

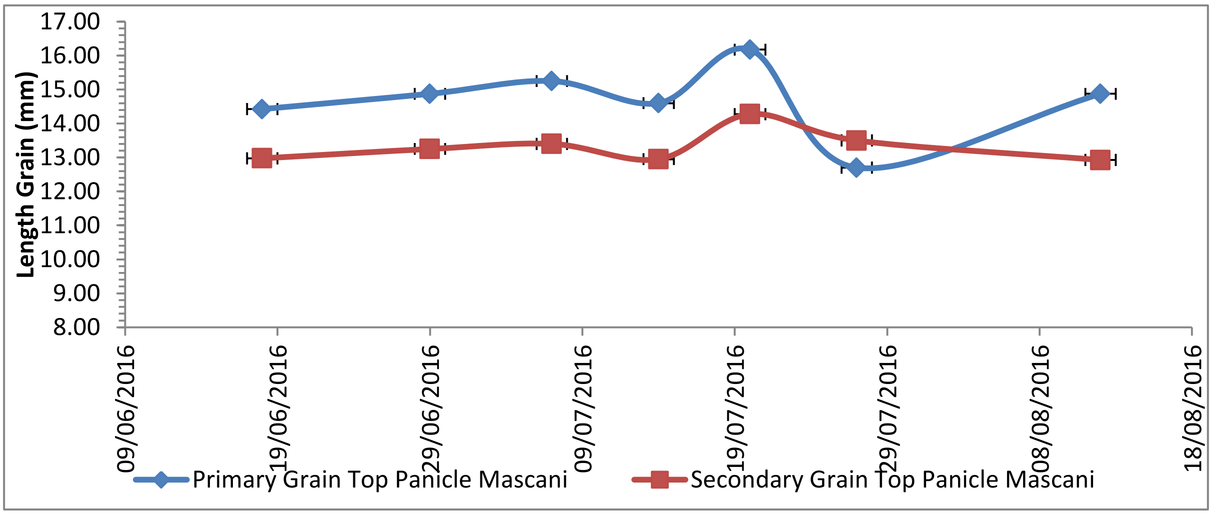


***Figure 5.23.a** Mean grain length (mm) ± s.e.m. throughout growth development of Mascani top panicle by primary and secondary grain.*

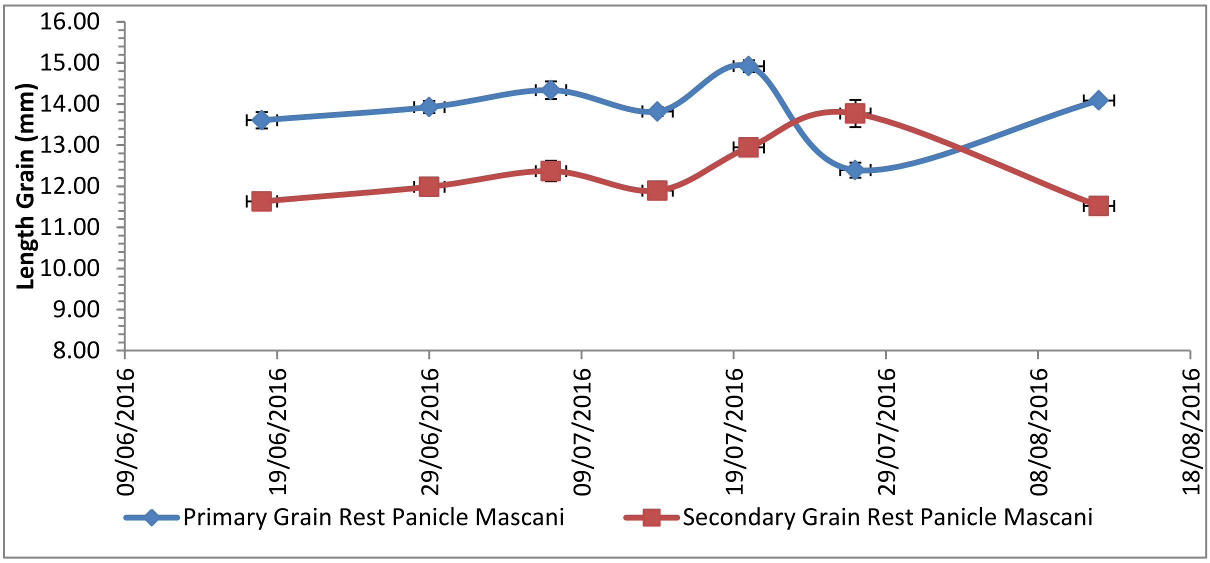


Figure 5.23.b *Mean grain length (mm) ± s.e.m. throughout growth development of Mascani rest of the panicle by primary and secondary grain.*

There were no substantial differences between the top and the rest of the panicle mean grain length (mm) (figure 5.24.a, b) for Tardis between early and final grain development . This effect was more evident for the rest of the panicle.

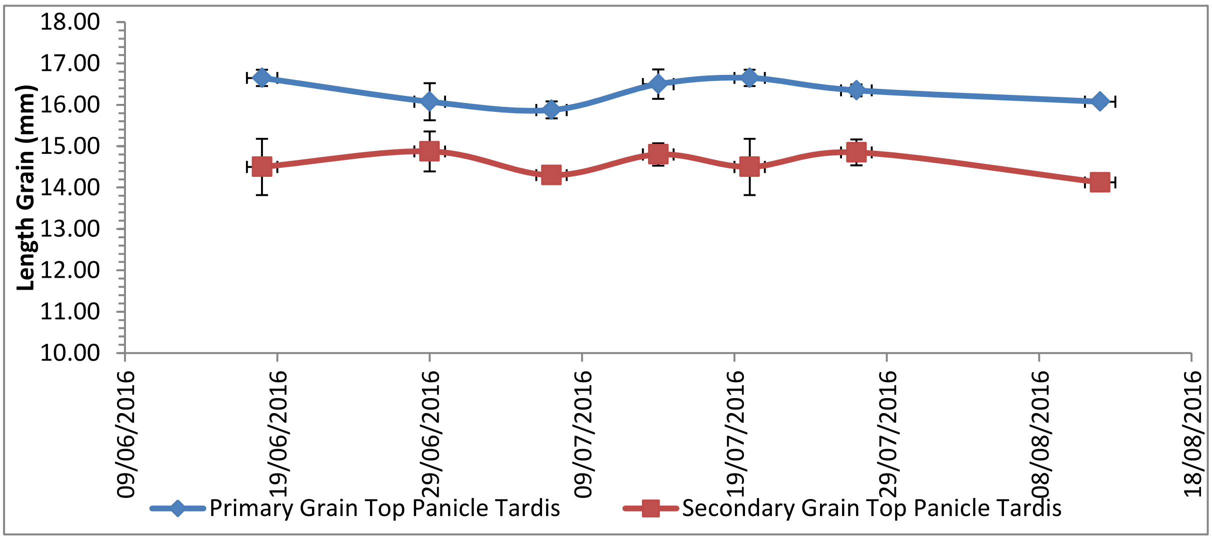


Figure 5.24.a *Mean grain length (mm) ± s.e.m. throughout growth development of Tardis top panicle by primary and secondary grain.*

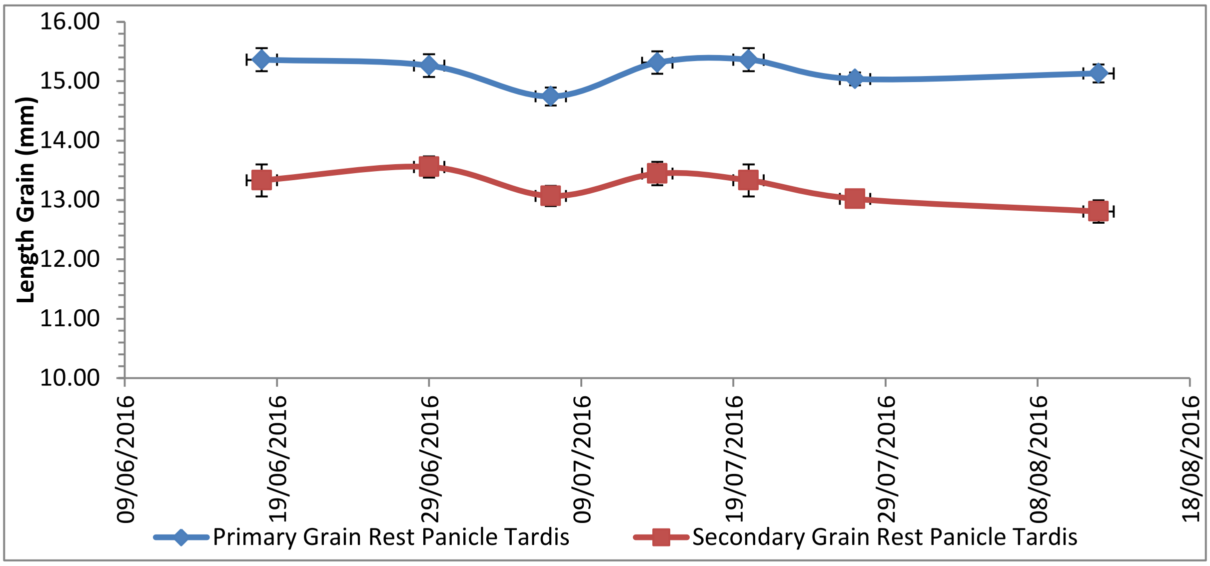


Figure 5.24.b *Mean grain length (mm) ± s.e.m. throughout growth development of Tardis rest of the panicle.*

The rate of change for primary and secondary grain length was calculated for each growth stage in comparison with the previous growth stage. Primary and secondary grain length in Buffalo showed similar rates of change pattern (figure 5.25 and 5.26). At late milk-soft dough (figure 5.25 and 5.26, point 2) maximum primary and secondary grain rates of growth were found. At the same time, both, primary and secondary grain showed lower rates of change at the top of the panicle. Secondary grain rate of change (figure 5.26), was lower than primary grain at the same growth stage (figure 5.25) e.g. -10% rate of change at whorl 3 secondary grain between soft dough and hard dough in comparison with a 0% primary grain rate of change at whorl 3 between soft dough and hard dough.

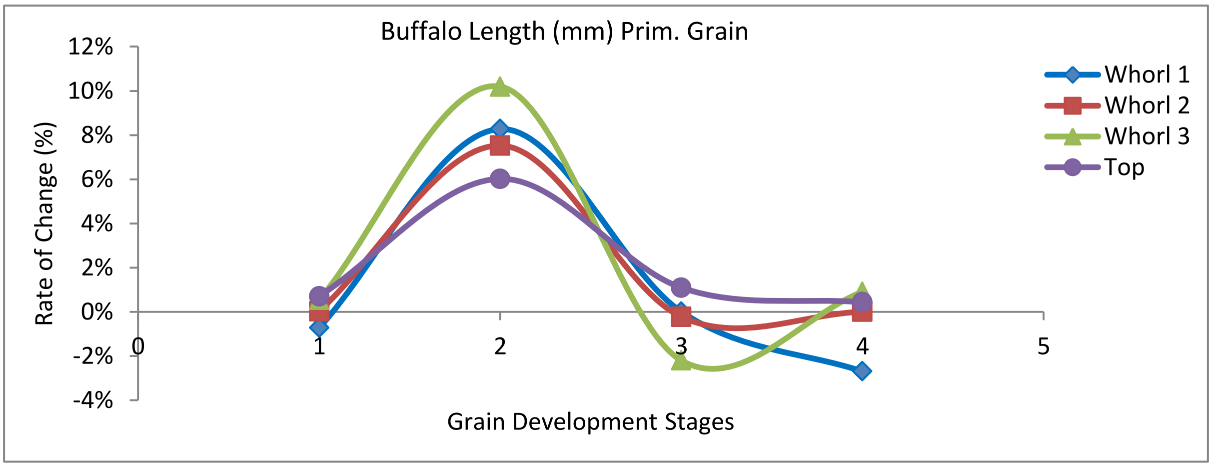


Figure 5.25 *Rate of change (%) for grain length (mm) values of primary grain by whorls between Buffalo growth development stages.*

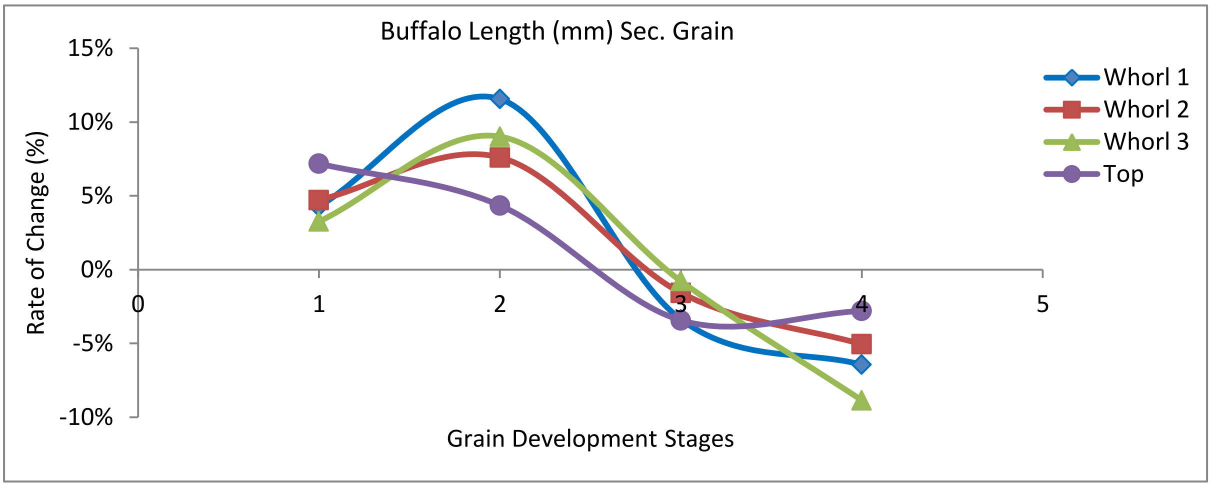


Figure 5.26 *Rate of change (%) for grain length (mm) values of secondary grain by whorls between Buffalo growth development stages.*

Primary and secondary grain length in Mascani had similar rates of change (figure 5.27 and 5.28). Maximum primary and secondary grain rates of change were found between soft dough-hard dough (figure 5.27 and 5.28 point 3). This was the opposite of the pattern found for Buffalo at the top of the panicle (figure 5.25 and 5.26). Mascani primary and secondary grain at the same position displayed higher rates of change when compared to the rest of whorls (figure 5.27 and 5.28). Secondary grain rates of change (figure 5.28) were lower than primary grain at the same growth stage (figure 5.27) with values below -8% rate of change at all whorls secondary grain between soft dough and hard dough in comparison with maximum rate of change of -7% primary grain rate of change at all whorls between soft dough and hard dough.

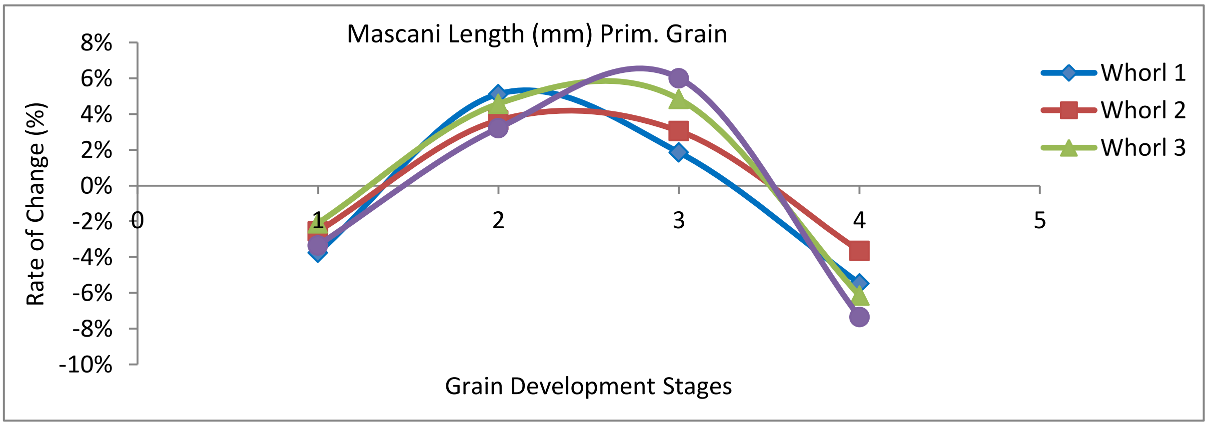


Figure 5.27 *Rate of change (%) for grain length (mm) values of primary grain by whorls between Mascani growth development stages.*

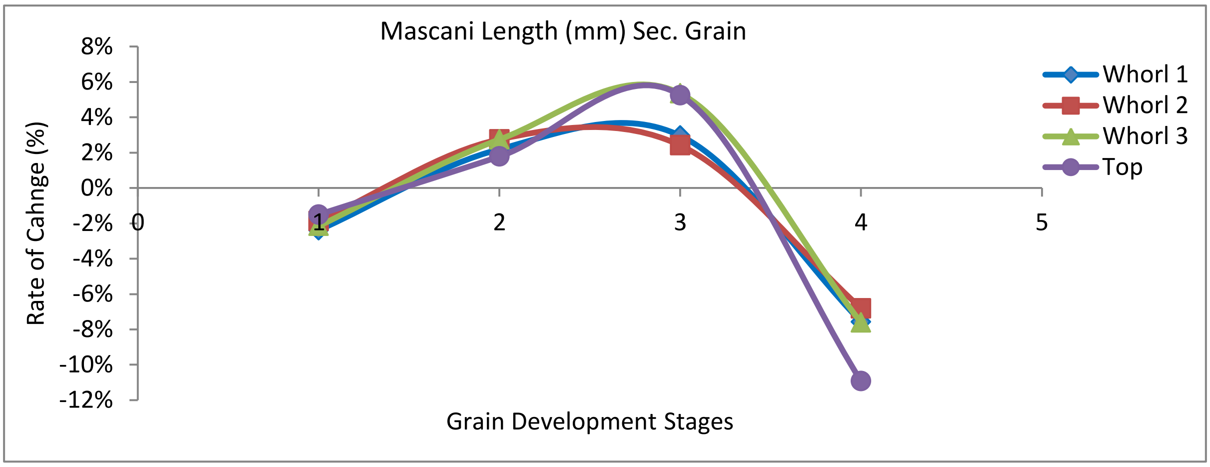


Figure 5.28 *Rate of change (%) for grain length (mm) values of secondary grain at each whorl between Mascani growth development stages.*

Primary and secondary grain length in Tardis had different rates of change pattern (figure 5.29 and 5.30). Maximum primary grain rate of growth was found between late milk-soft dough (figure 5.29 point 2) but only at the top and whorls 1 and 2 whorls. At the bottom of the panicle, whorl 1 primary grain showed a different pattern of rate of change being higher between soft dough-hard dough. At the same time, primary grain had different rates of change between top and whorl 1 and whorls 2 and 3. Secondary grain rates of change (figure 5.30) were higher at soft dough-hard dough and rates of change were strongly negative at final stages of grain development, e.g. -12% at all whorls (figure 5.30 point 4) when compared to primary grain growth rate at the same growth stage.

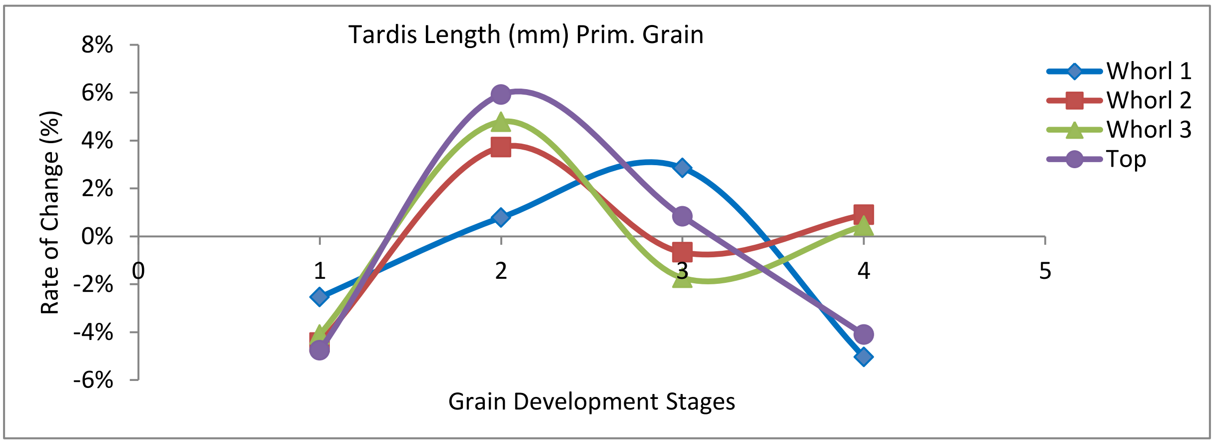


Figure 5.29 *Rate of change (%) for grain length (mm) values whorls of primary grain at each whorl between Tardis growth development stages.*

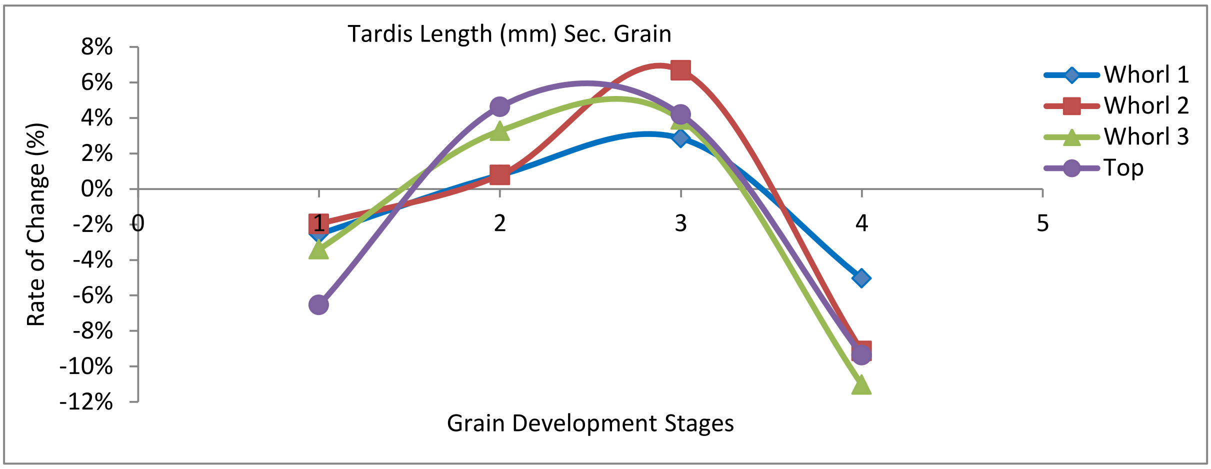


Figure 5.30 *Rate of change (%) for grain length (mm) values whorls of secondary grain at each whorl between Tardis growth development stages.*

5.3.4.3 Grain Width

Mean grain width (mm) displayed statistically significant differences (p-value<0.001, MANOVA) between growth stages (figure 5.31). There were statistical significant differences between varieties, type of grain and significant interactions between variety and growth stage and between type of grain and growth stage were found (p-value<0.001).

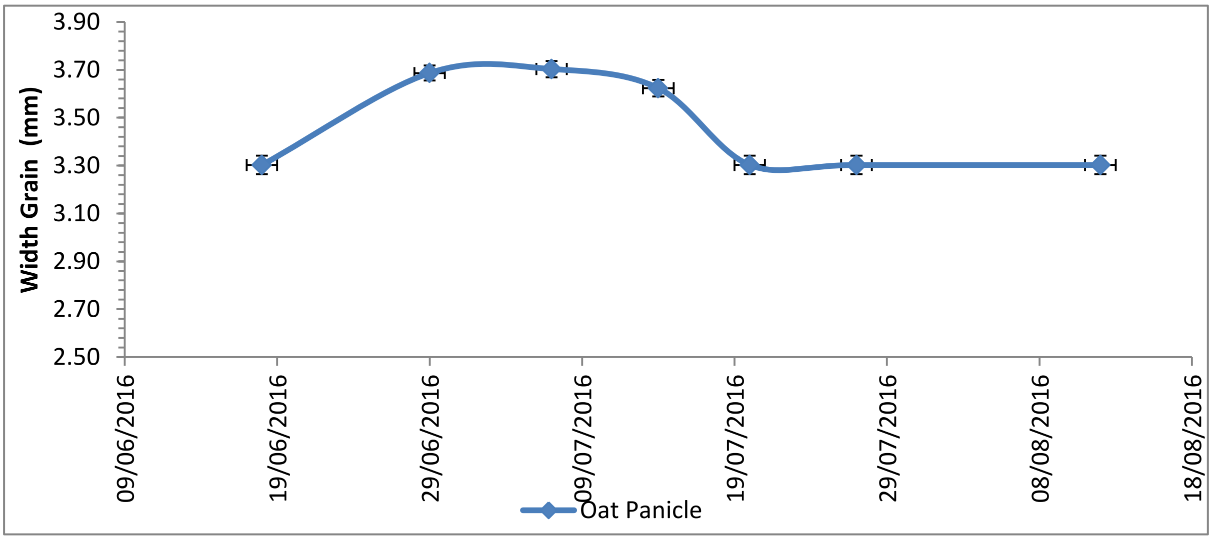


Figure 5.31 *Mean grain width (mm) ± s.e.m. throughout grain development stages of Buffalo, Mascani and Tardis.*

Similar pattern was found when comparing the top and the rest of the panicle grain width. Higher values were found in both cases at late milk (figure 5.32.a and 5.32.b), decreasing at the final stages reaching lower values compared to early stages. There were no substantial differences in primary and secondary grain width pattern.

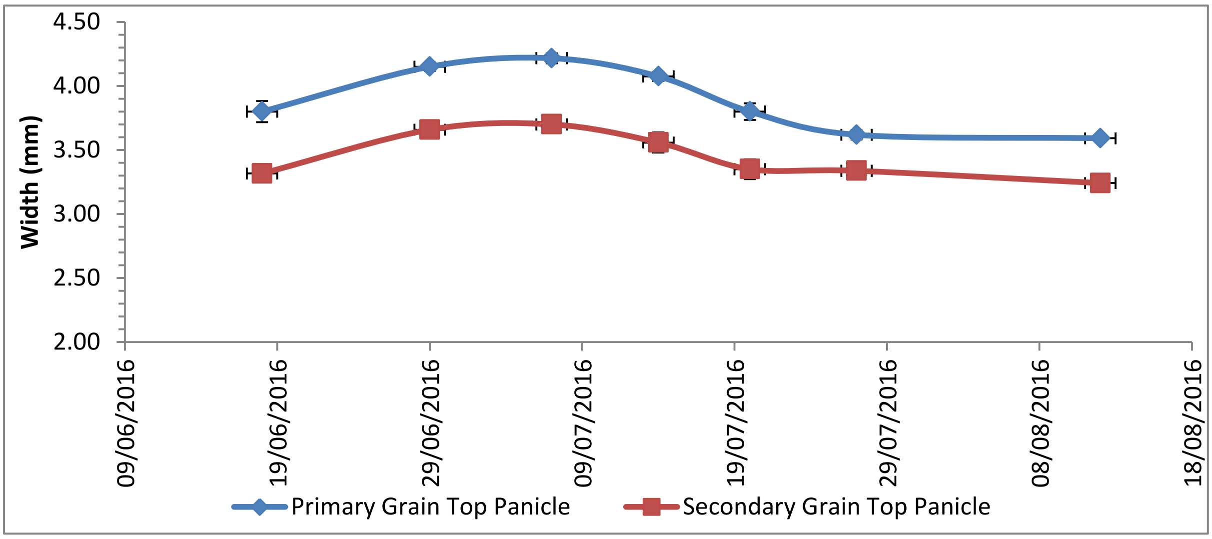


Figure 5.32.a *Mean grain width (mm) ± s.e.m. by primary and secondary grain throughout grain development stages of Buffalo, Mascani and Tardis, top panicle.*

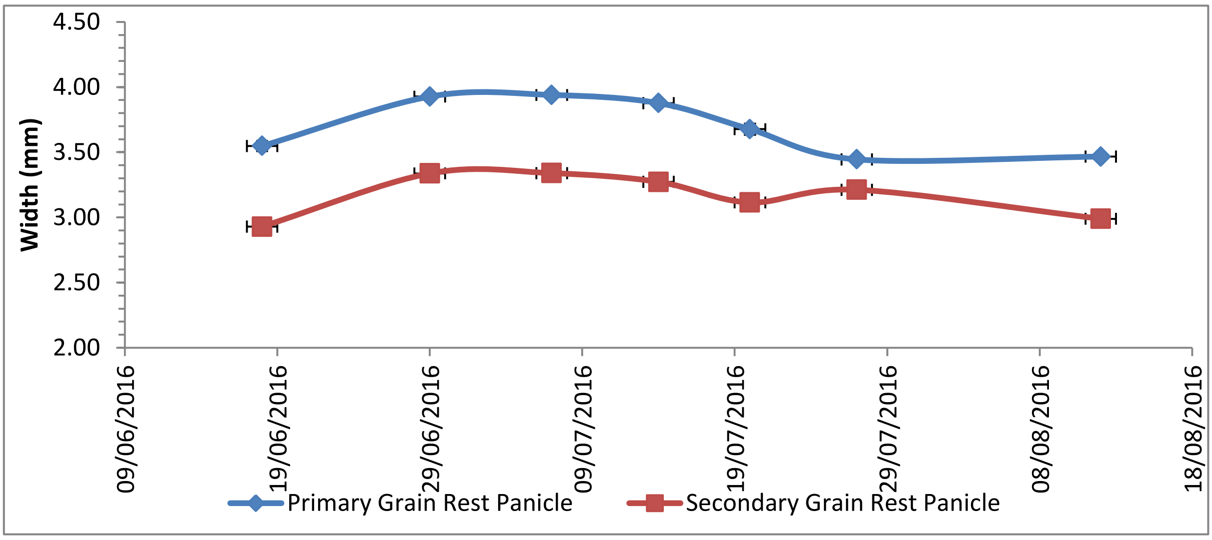


Figure 5.32.b *Mean grain width (mm) ± s.e.m. by primary and secondary grain throughout grain development stages of Buffalo, Mascani and Tardis, rest of panicle.*

Buffalo grain width was higher at late milk (30/06/2016) at the top and the rest of whorls in the panicle (figure 5.33.a and 5.33.b). Both, the top and the rest of whorls grain width, decreased at the end of growth development.

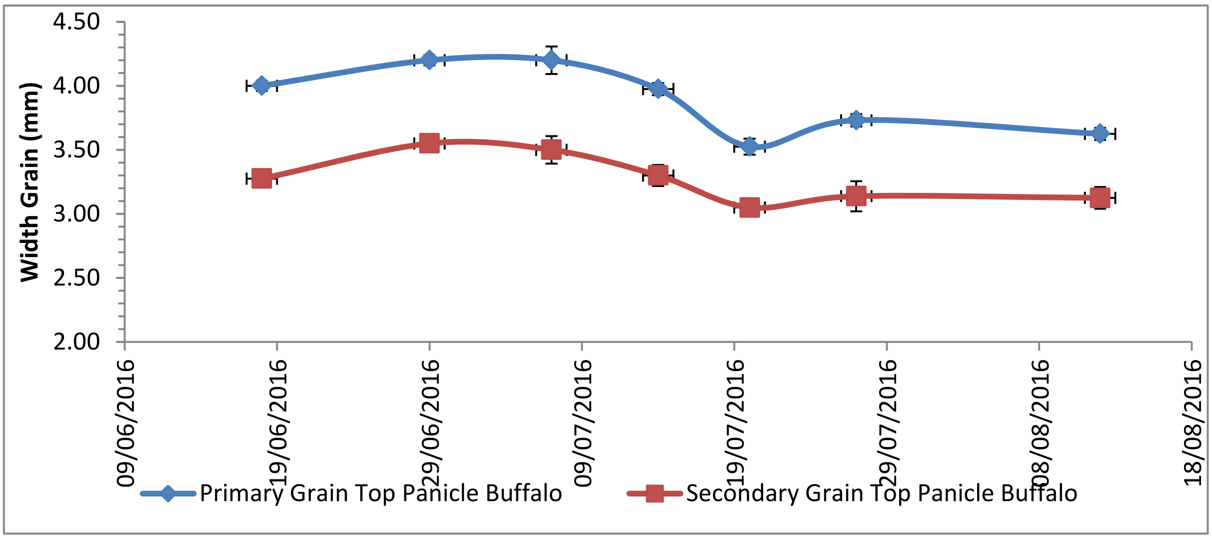


Figure 5.33.a *Mean grain width (mm) ± s.e.m. by primary and secondary grain along growth development stages of Buffalo top panicle.*

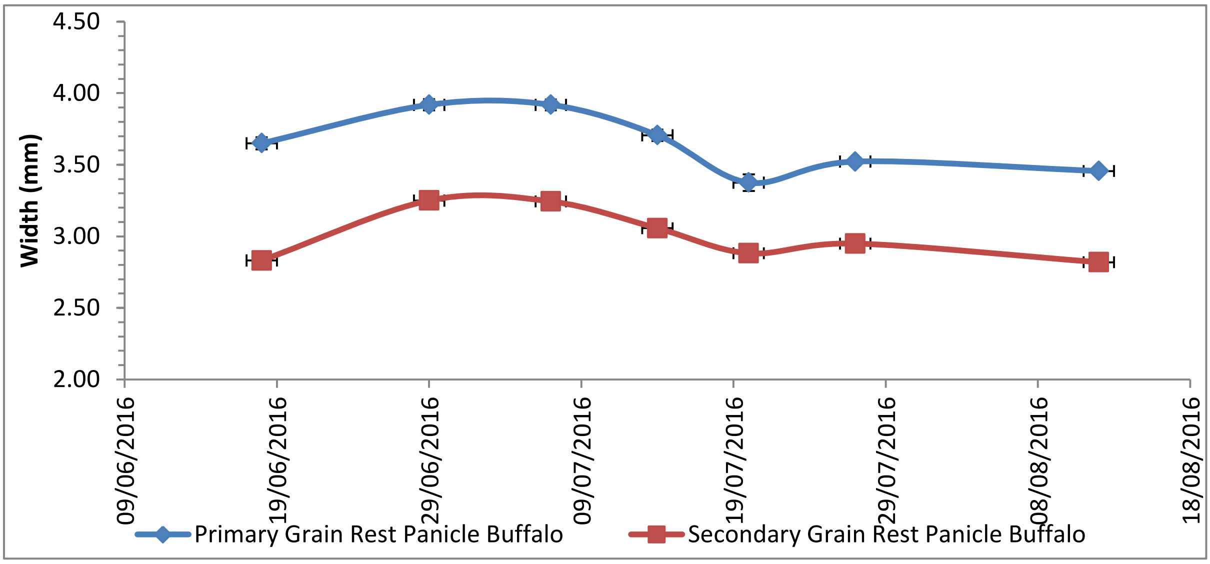


Figure 5.33.b *Mean grain width (mm) ± s.e.m. by primary and secondary grain along growth development stages of Buffalo rest of panicle.*

Mascani mean grain width was higher at late milk (30/06/2016) at the top of the panicle (figure 5.34.a) whilst the rest of whorls reached highest grain width at soft dough (figure 5.34.b). The differences in mean grain width for the entire panicle between early milk, 3.52mm, and hard dough, 3.23 mm, were greater in Mascani compared to the other two varieties, Buffalo, 3.27mm at early milk and 3.27 at hard dough, and Tardis 3.14 mm at early milk and 3.17 at the end of grain development. There was an overlap grain primary and secondary width time pattern between soft dough and hard dough at the top of the panicle (figure 5.34.a) while at the rest of the panicle secondary grain width was higher than primary grain between soft dough and hard dough (5.34.b).

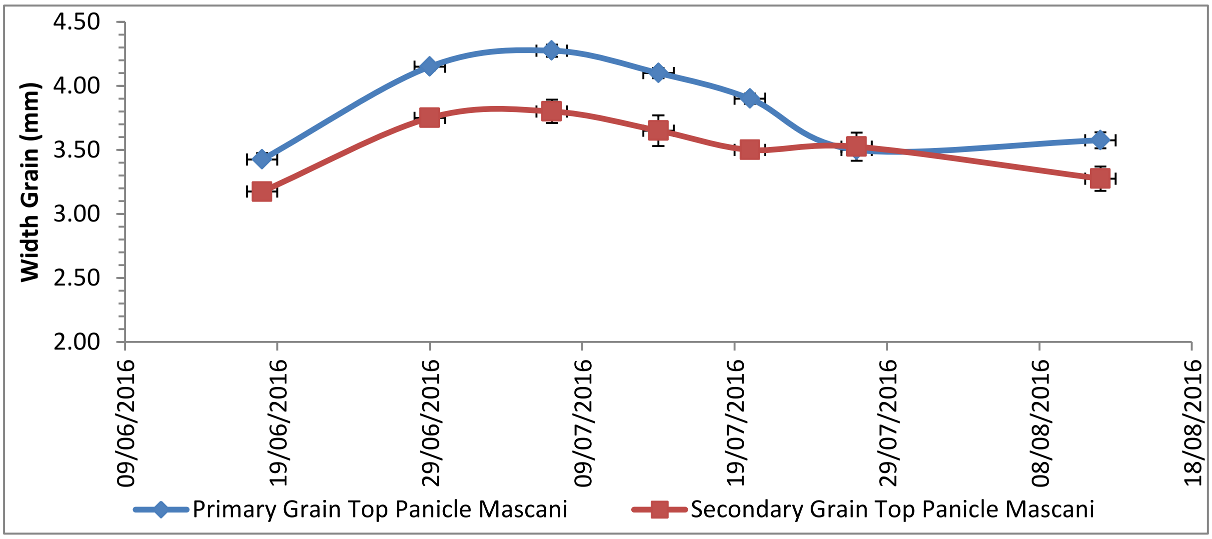


Figure 5.34.a *Mean grain width (mm) ± s.e.m. by primary and secondary grain along grain development stages Mascani top panicle.*

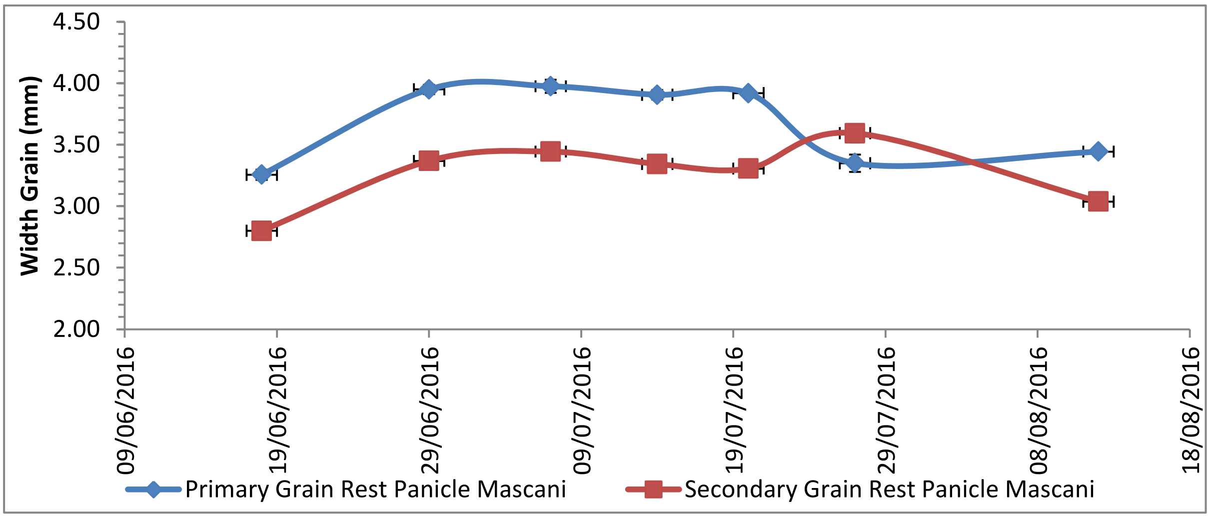


Figure 5.34.b *Mean grain width (mm) ± s.e.m. by primary and secondary grain throughout grain development stages Mascani rest of panicle.*

Tardis grain width was highest at late milk (30/06/2016) at the top panicle (figure 5.35.a) while at the rest of the panicle (figure 5.35.b) had highest between late milk and soft dough. From that point, grain width decreased at all whorls and showed no change until hard dough.

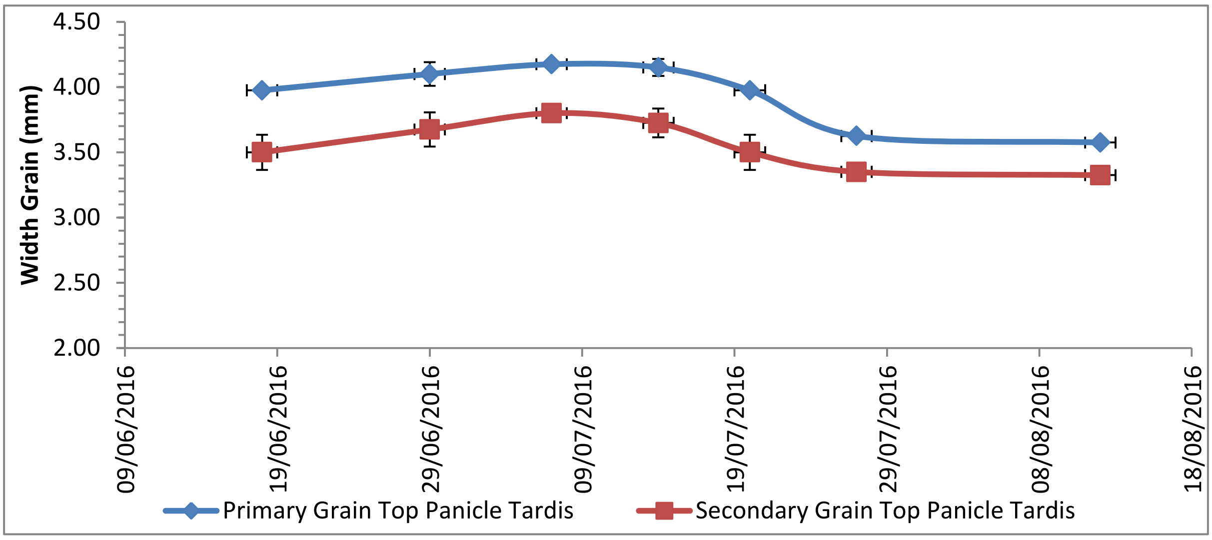


Figure 5.35.a *Mean grain width (mm) ± s.e.m. by primary and secondary grain along growth development stages of Tardis top panicle.*

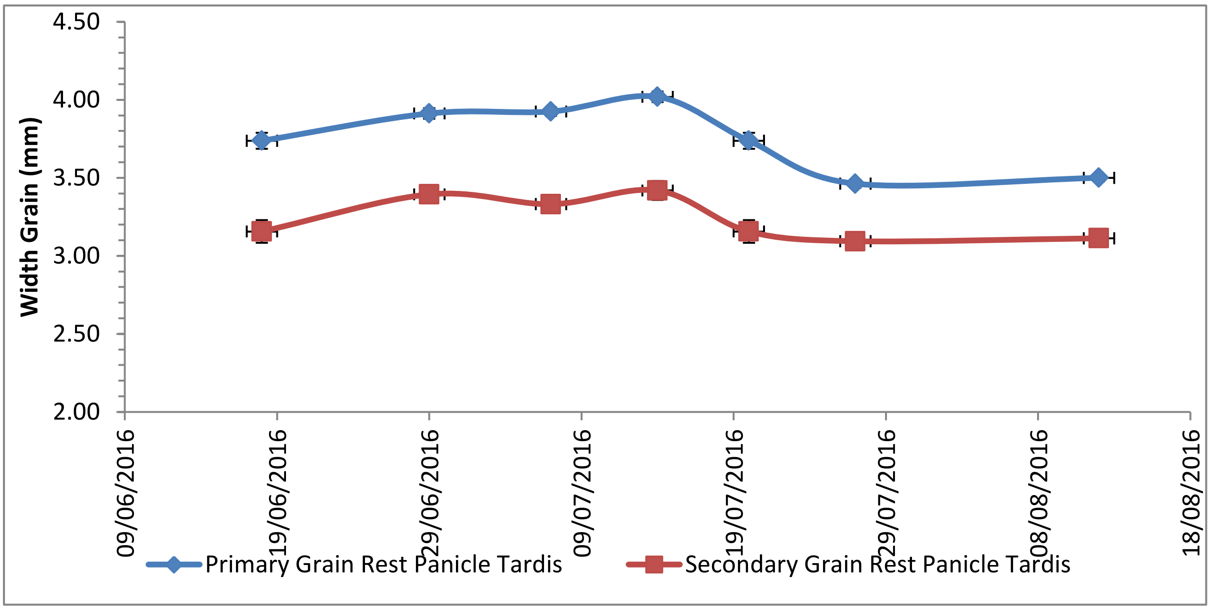


Figure 5.35.b *Mean grain width (mm) ± s.e.m. by primary and secondary grain along growth development stages of Tardis rest of panicle*.

Rate of change in grain width (table 5.6) was higher for primary and secondary grain in the lowest whorls when compared to the top of the panicle for all varieties. Secondary grain rate of change was higher than primary grain change rate for all varieties and between all growth stages, with higher increases between first stages of development and higher reductions, i.e. negative rates of change, at the final stages of development.

Table 5.6 *Rate of change of mean grain width (mm) values and days between growth development stages of each variety primary and secondary grain.*

			Whorl 1		*Whorl 2*		*Whorl 3*		*Top*	
Variety		*Days*	*Primary*	*Secondary*	*Primary*	*Secondary*	*Primary*	*Secondary*	*Primary*	*Secondary*
Buffalo	***EM/LM***	10	6%	16%	5%	13%	4%	10%	2%	5%
	LM/SD	12	9%	12%	8%	11%	7%	9%	3%	2%
	SD/HD	7	0%	-8%	-3%	-7%	-1%	-5%	-5%	-3%
	HD	14	-15%	-5%	-12%	-6%	1%	-8%	-4%	-6%
Mascani	***EM/LM***	14	-3%	9%	-1%	6%	-1%	3%	-5%	-3%
	LM/SD	13	6%	5%	2%	3%	2%	2%	-3%	4%
	SD/HD	5	3%	1%	2%	2%	3%	3%	0%	-5%
	HD	16	-14%	-12%	-11%	-10%	-13%	-13%	-9%	-9%
Tardis	***EM/LM***	12	14%	15%	11%	17%	9%	7%	7%	7%
	LM/SD	13	5%	5%	1%	-2%	-3%	0%	-6%	-6%
	SD/HD	5	-4%	-5%	1%	1%	0%	1%	3%	0%
	HD	16	-8%	-1%	-10%	-5%	-9%	-8%	-10%	-6%

5.3.4.4 Groat area

The analysis described above was repeated after all grain samples had been manually dehulled. Mean groat area values showed significant differences between growth stages (figure 5.36), varieties and type of groat (p-value<0.001). Significant interactions were found between variety and growth stage and between growth stage and type of groat (p-value<0.001).

Groat area throughout development had a plateau (figure 5.36) between late milk and soft dough, decreasing abruptly at the end of groat development to similar values to early stages.

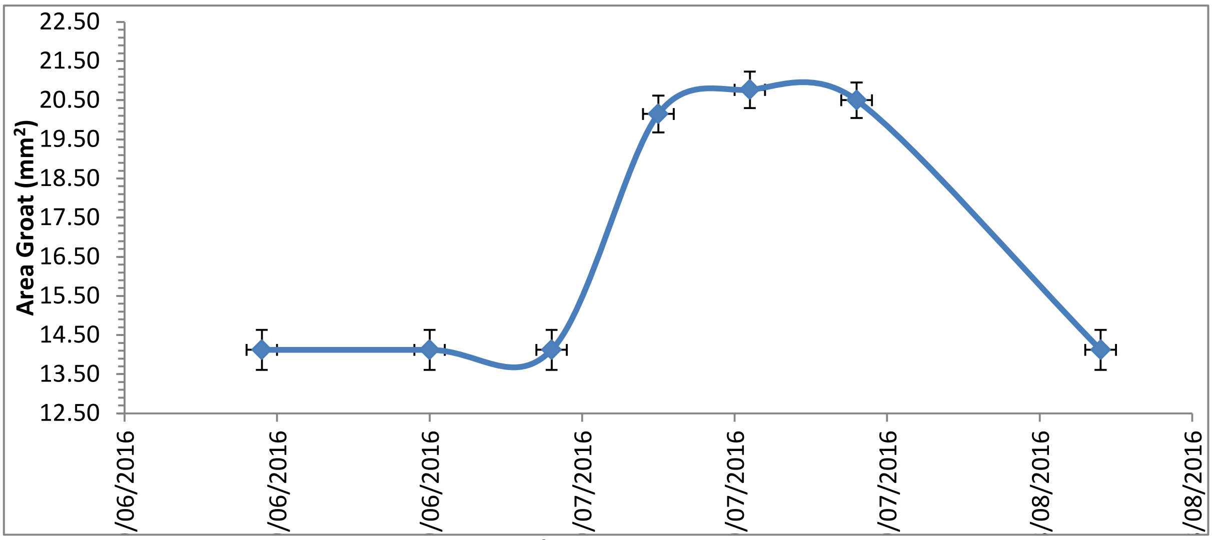


***Figure 5.36** Mean groat area (mm^2) ± s.e.m. throughout groat development of Buffalo, Mascani and Tardis panicle.*

However, groat area time pattern changed when analysed by primary and secondary grain and at the top and the rest of whorls in the panicle. Highest groat area was between early milk and late milk (figure 5.37), showing similar pattern at top panicle and all whorls and between primary and secondary grain.

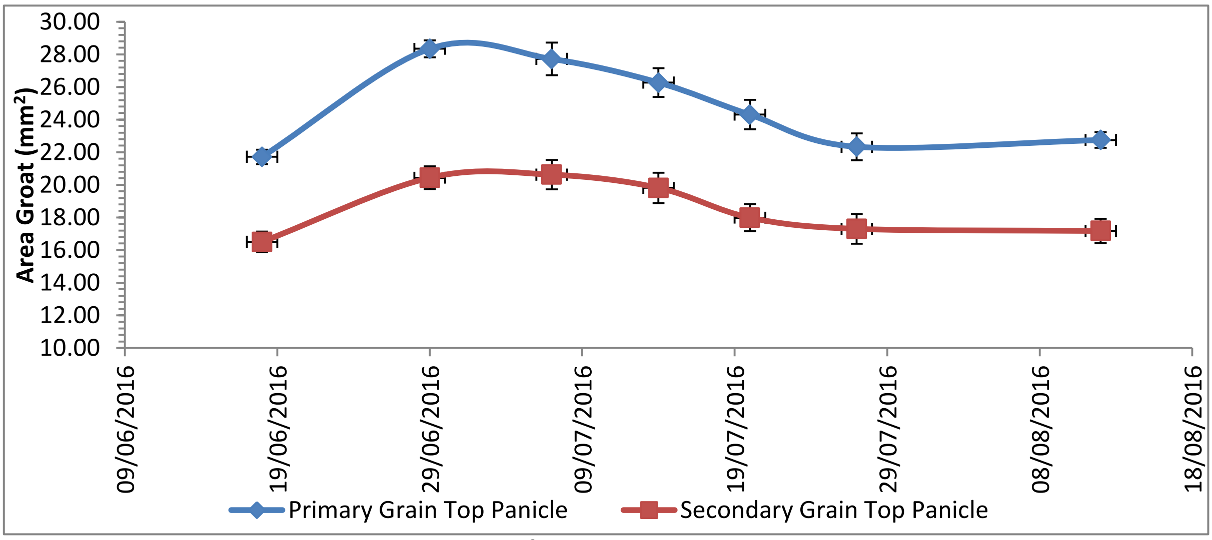


***Figure 5.37** Mean groat area (mm^2) ± s.e.m. by primary and secondary groat throughout grain development of Buffalo, Mascani and Tardis panicle.*

Buffalo, Mascani and Tardis mean groat areas (mm^2) top panicle and rest of whorls showed statistical differences in mean values but the similar pattern of development. Thus, at late milk both, primary and secondary groat reached the maximum Buffalo and Tardis groat area, whilst between late milk and soft dough for Mascani. Minimum mean groat area was at early milk for the three varieties (figure 5.38, 5.39, 5.40) and at hard dough in Mascani and Tardis (figure 5.39 and 5.40). At the top of the panicle mean groat area values were always higher when compared to other whorls.

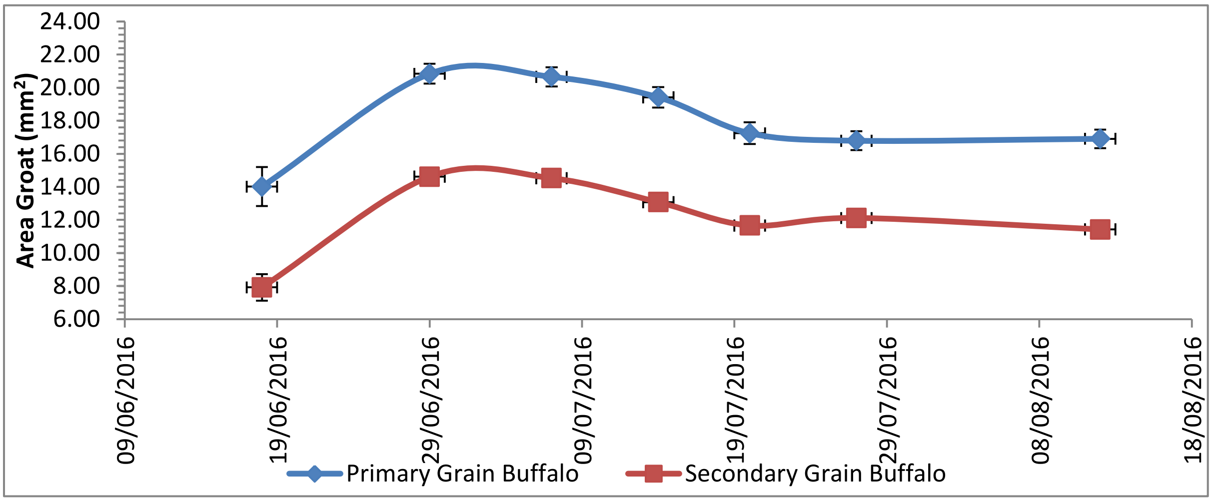


Figure 5.38 *Mean groat area (mm^2) ± s.e.m. by primary and secondary groat along growth development stages of Buffalo panicle.*

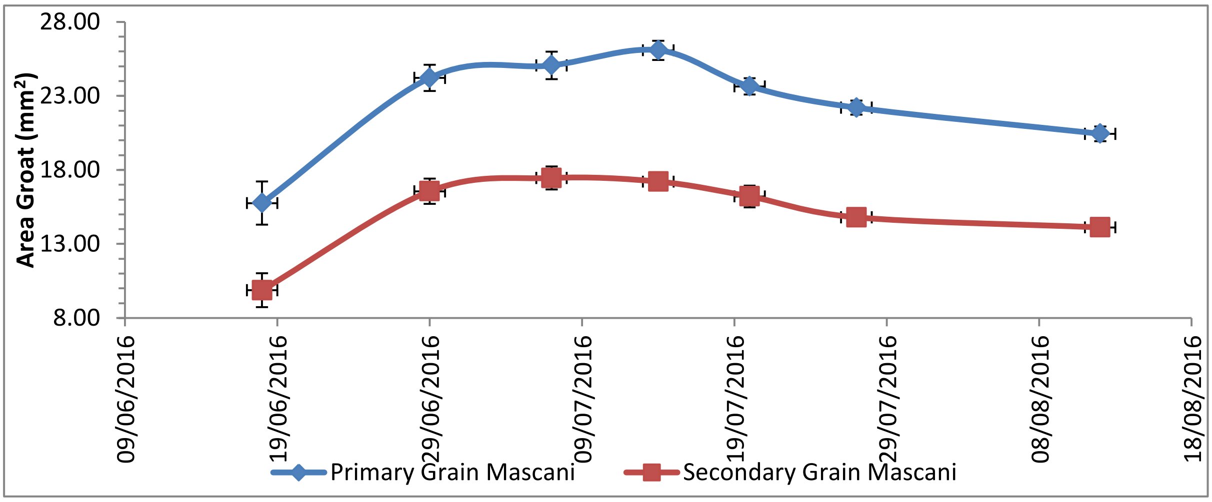


Figure 5.39 *Mean groat area (mm^2) ± s.e.m. by primary and secondary groat along growth development stages of Mascani panicle.*

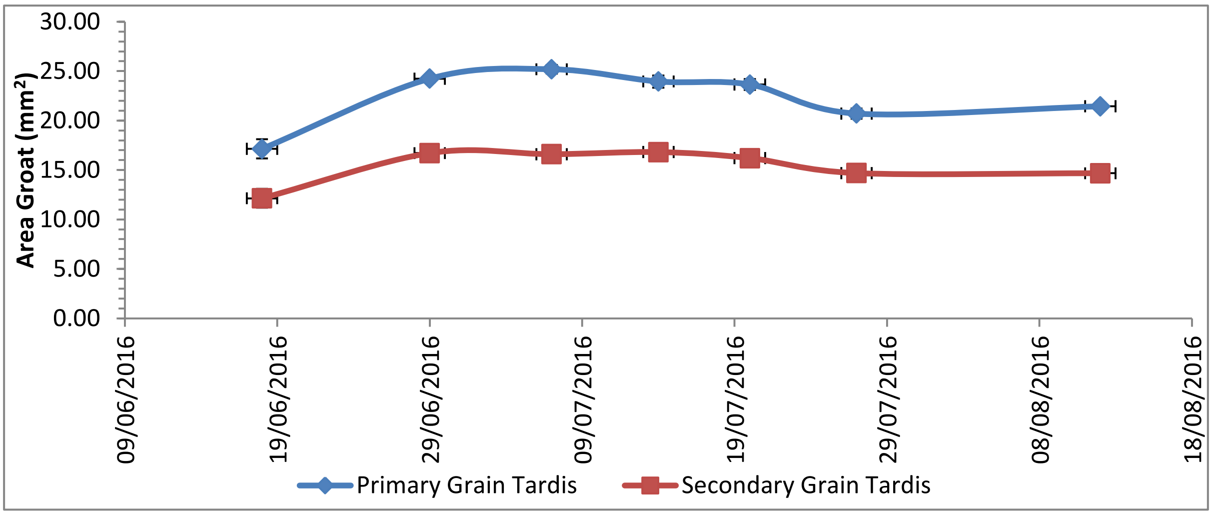


Figure 5.40 *Mean groat area (mm^2) ± s.e.m. by primary and secondary groat along growth development stages of Tardis panicle*

Top panicle groat width mean values, when analysed by primary and secondary grain had the same patterns for each variety as those for whorls show above (figure 5.38, 5.39 and 5.40).

5.3.4.5 Groat width

Mean groat width (mm) values showed significant differences between growth stages (figure 5.41), whorls, varieties, and type of groat (p-value<0.001). Significant interactions were found between variety and growth stage and between variety and type of groat (p-value<0.001).

Groat width throughout development had a plateau (figure 5.41) between early and late milk and soft dough, decreasing abruptly at the end of groat development to similar values to early stages with similar pattern found for groat area (figure 5.36).

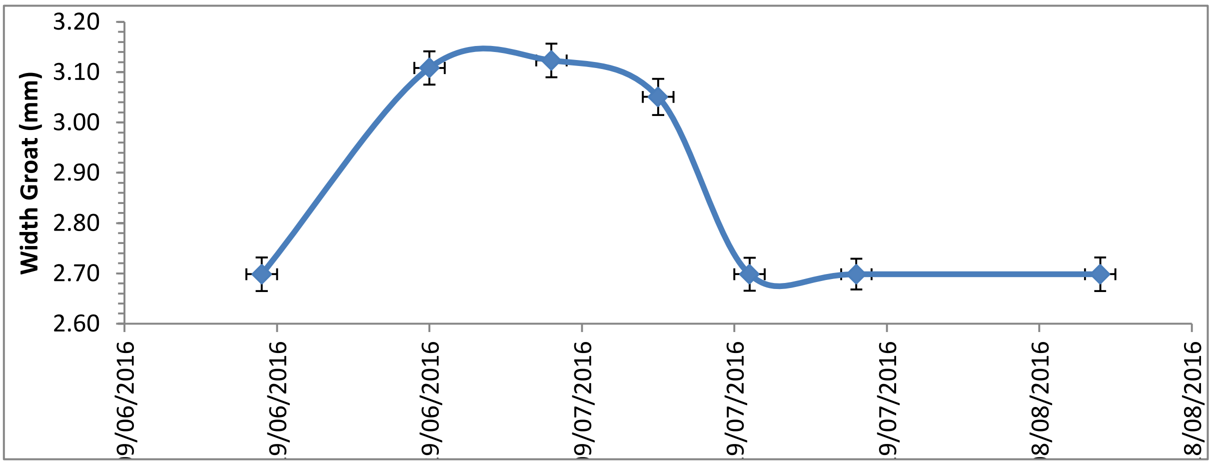


Figure 5.41 *Mean groat width (mm) ± s.e.m. throughout groat development of Buffalo, Mascani and Tardis panicle.*

Buffalo and Tardis mean groat width (mm) (figure 5.42, 5.44) was greatest between early and late milk decreasing at soft dough without any further significant change until hard dough. Mascani mean groat width (mm) by whorl (figure 5.43) reached higher values between late milk and soft dough, showing minimum values the end and beginning of the groat development.

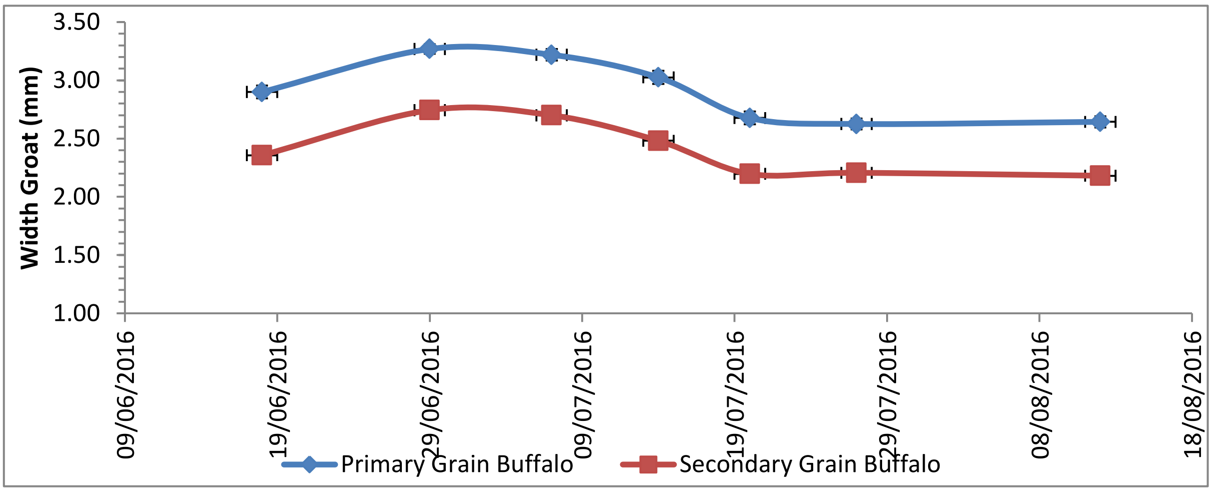


Figure 5.42 *Mean groat width (mm) ± s.e.m. by primary and secondary groat throughout Buffalo groat development.*

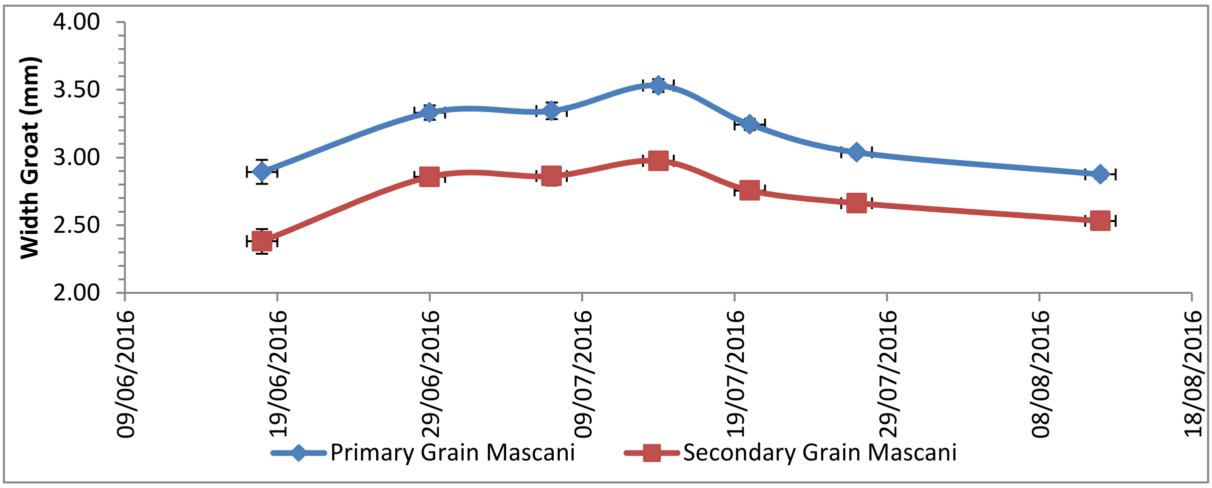


Figure 5.43 *Mean groat width (mm) ± s.e.m. by primary and secondary groat throughout Mascani groat development.*

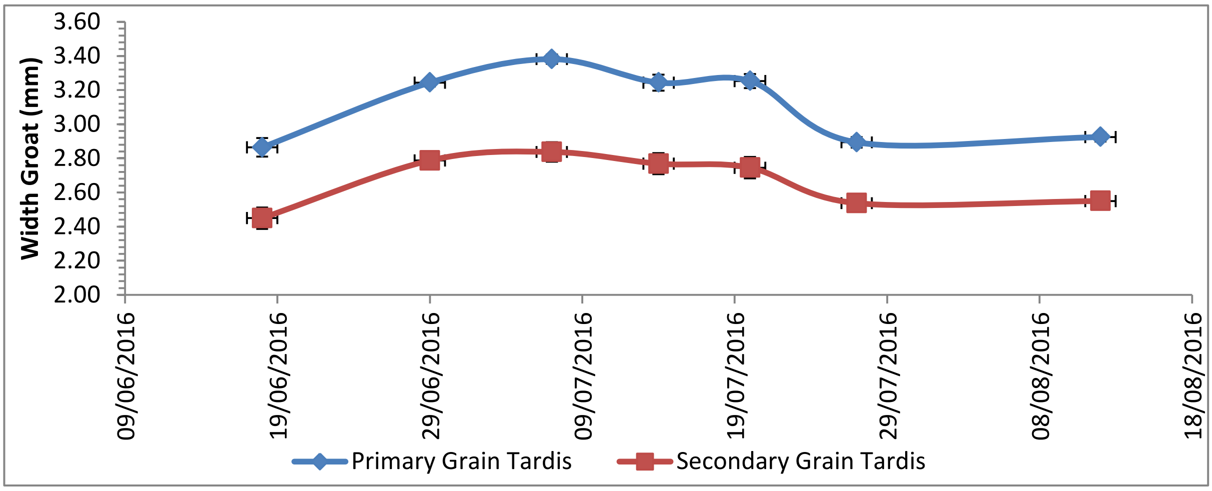


Figure 5.44 *Mean groat width (mm) ± s.e.m. by primary and secondary groat throughout Tardis groat development.*

Top panicle groat width mean values, when analysed by primary and secondary grain had the same patterns for each variety as those for the whorls shown above (figure 5.42, 5.43 and 5.44).

5.3.4.6 Groat length

Mean groat length (mm) showed significant differences between growth stages (figure 5.45) whorls, varieties and type of groat (p-value<0.001). A significant interaction was also found between variety and growth stage.

Groat length throughout development had maximum and a plateau (figure 5.45) between late milk and soft dough, decreasing abruptly at the end of groat development to similar values to early stages.

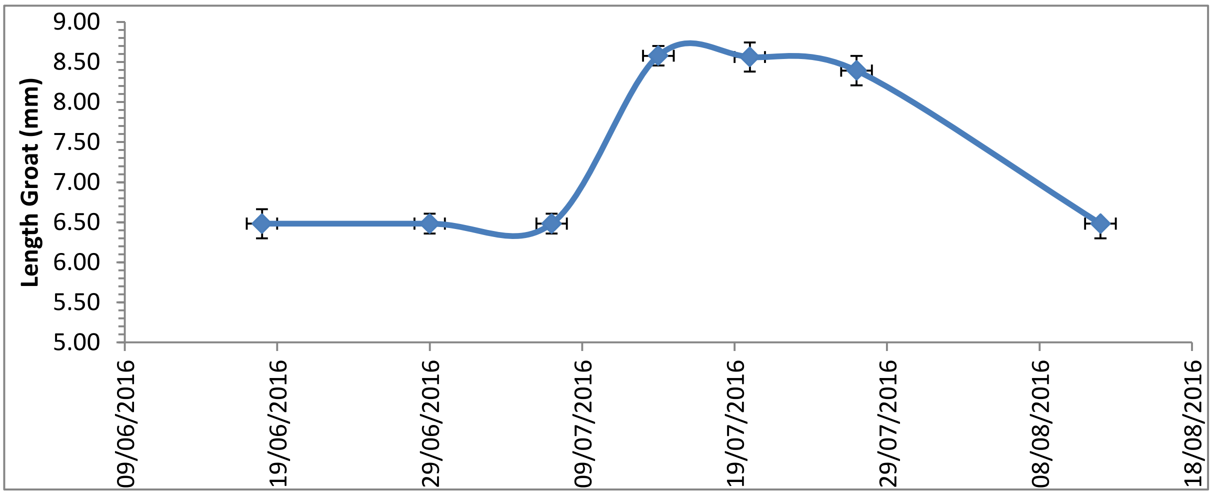


Figure 5.45 *Mean groat length (mm) ± s.e.m. throughout groat development of Buffalo, Mascani and Tardis panicle.*

Buffalo, Mascani and Tardis mean groat length (mm) (figure 5.46, 5.47 and 5.48) showed the same pattern of development. The highest point was reached at late milk with no significant change in mean length groat values until hard dough.

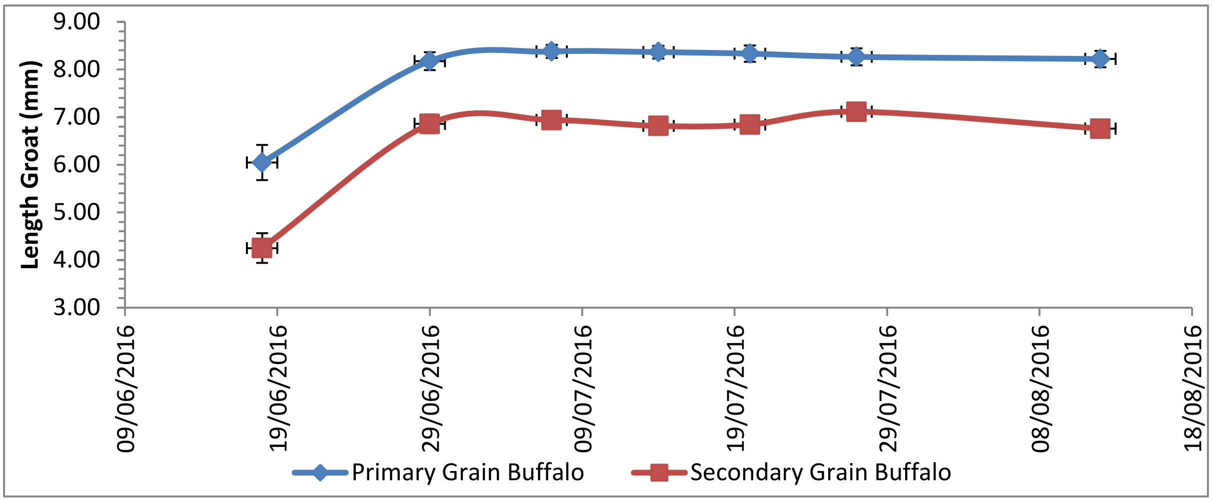


Figure 5.46 *Mean groat length (mm) ± s.e.m. by primary and secondary groat throughout Buffalo groat development.*

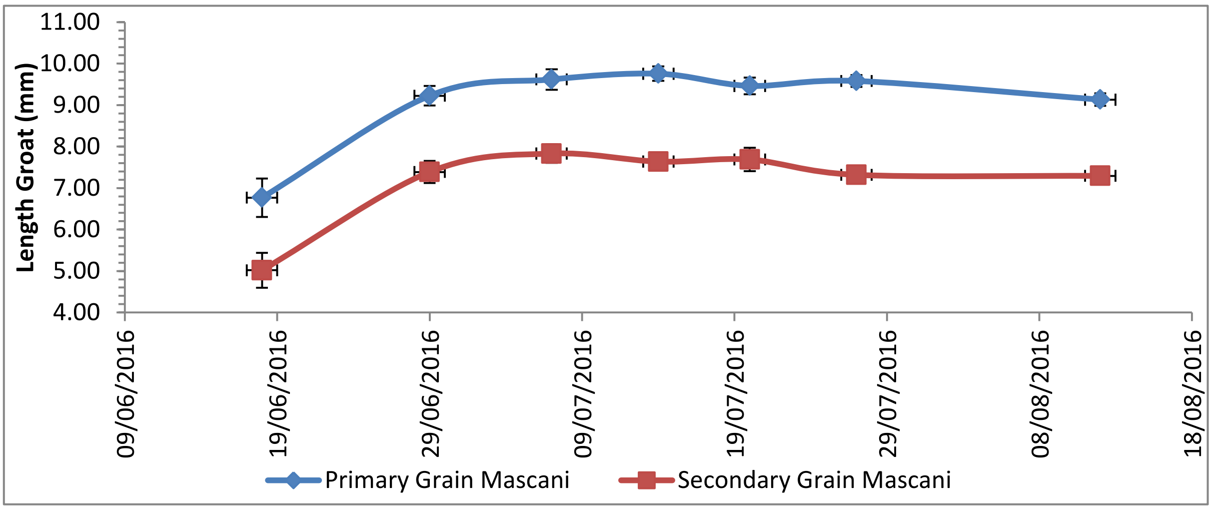


Figure 5.47 *Mean groat length (mm) ± s.e.m. by primary and secondary groat throughout Mascani groat development.*

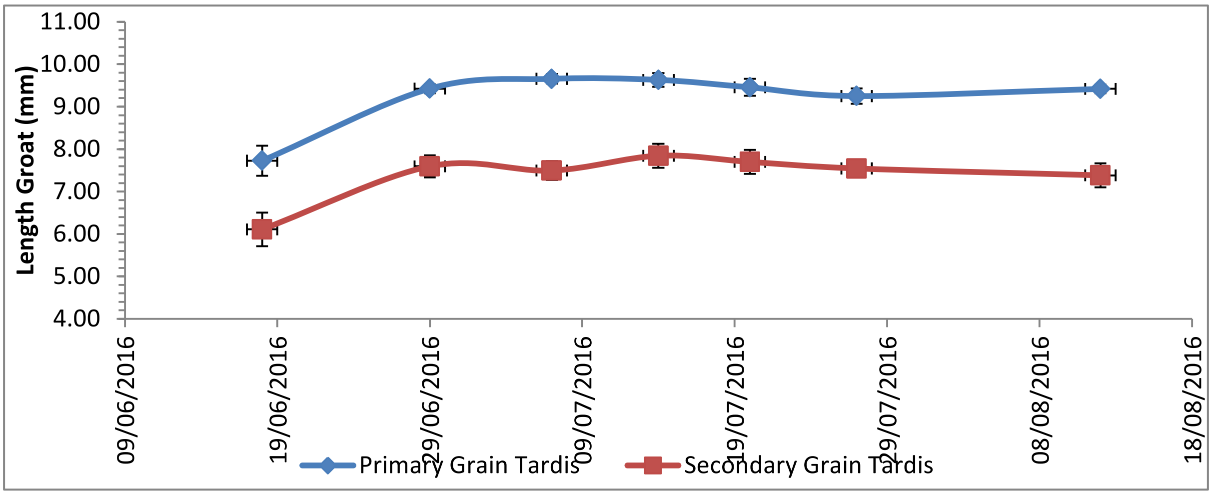


Figure 5.48 *Mean groat length (mm) ± s.e.m. by primary and secondary groat throughout Tardis groat development.*

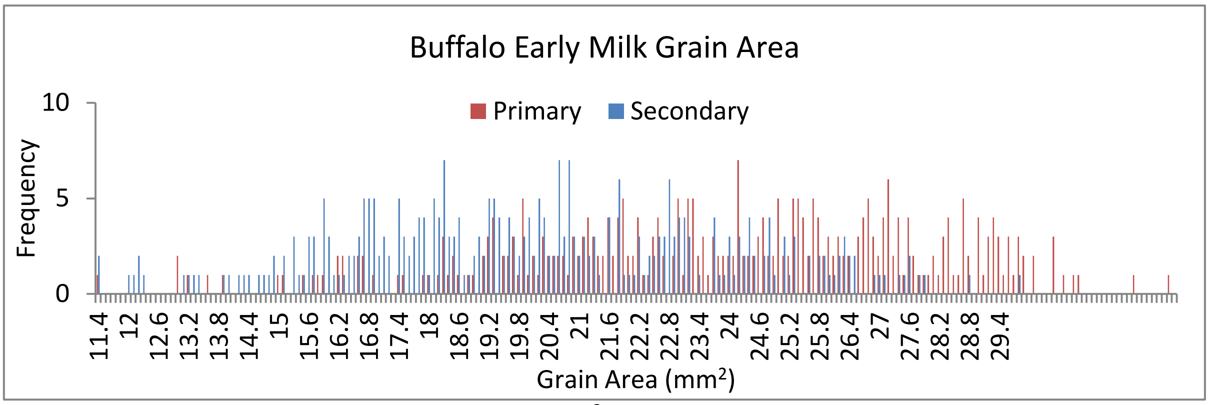
Top panicle groat length mean values, when analysed by primary and secondary grain had the same patterns for each variety as those for the whorls shown above (figure 5.46, 5.47 and 5.48).

5.3.5 Analysis of grain and groat size distribution

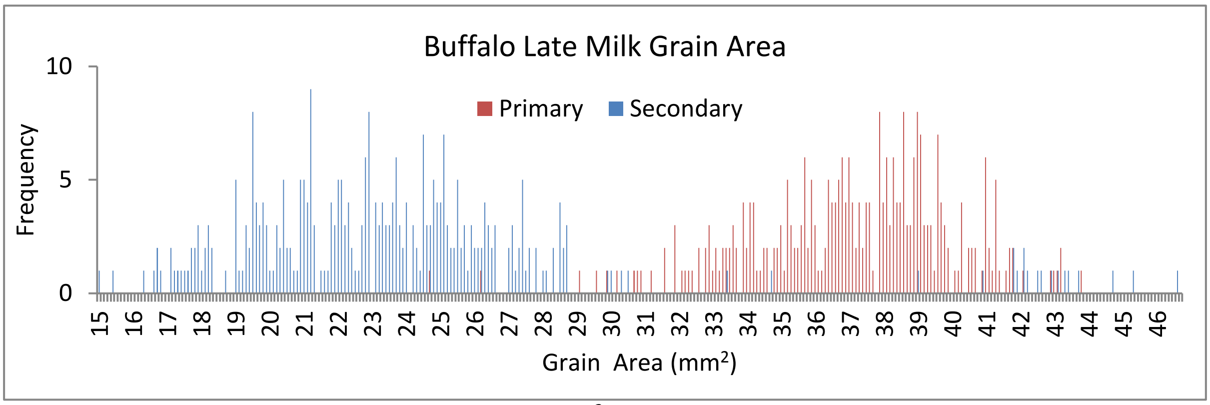
Final analysis included frequency distribution graphs on grain and groat size throughout grain development for the three varieties under study and for both primary and secondary grain.

5.3.5.1 Grain size

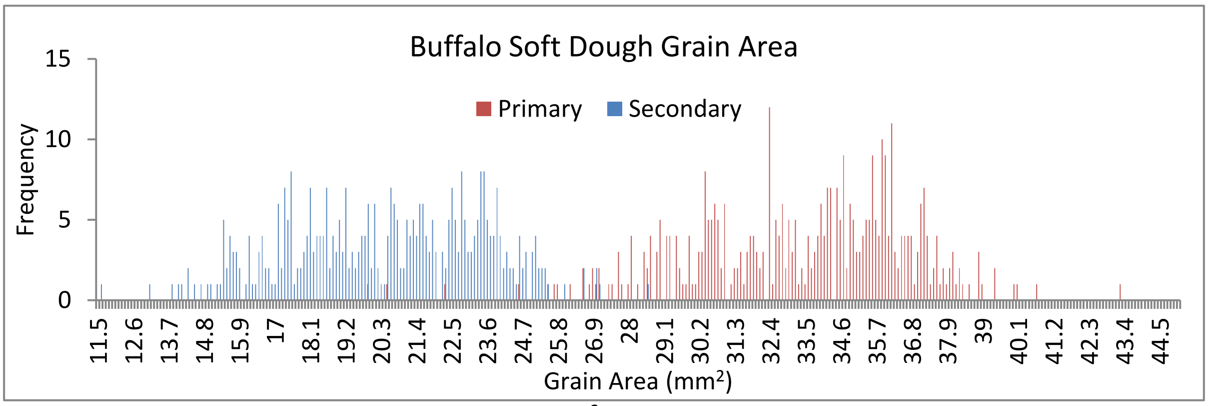
As an example figures 5.49 a to e show a frequency distribution of the grain area of Buffalo primary and secondary grain at different stages of development. Although the number of grains might vary between growth stages, a clear bimodal distribution was found from late milk. Late milk and soft dough showed higher frequencies at higher values of grain area for both primary and secondary grain and a clearer bimodal distribution. The boundaries between primary and secondary grain area were not so clear at both early milk and hard dough stages. Tertiary grain was only found in samples taken at hard dough, when, primary and secondary overlap was larger in comparison with earlier stages. These frequency distributions graphs clearly show graphically this reduction in the dispersion of the data. Similar results were found for Mascani and Tardis grain area (see appendix).



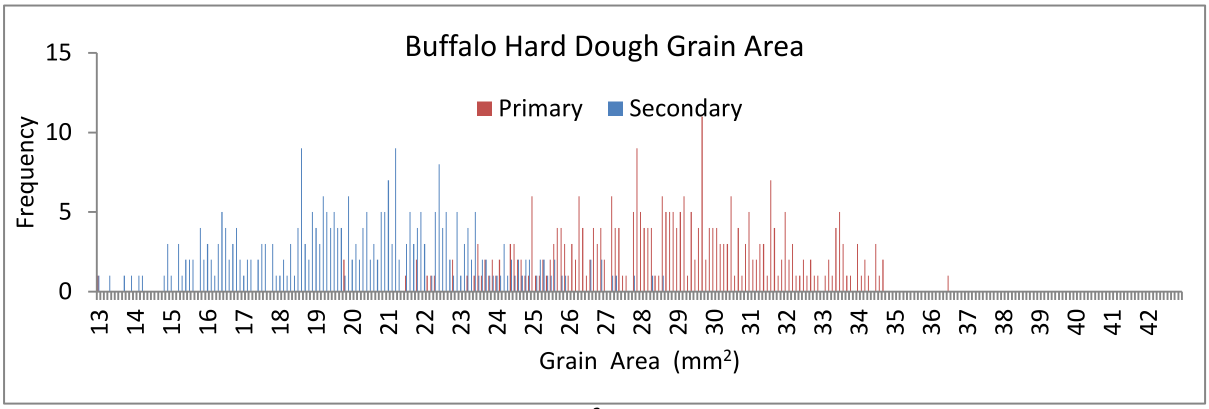
Figures 5.40.a *Frequency grain area (mm²) histograms throughout grain development stages Buffalo primary and secondary grain.*



Figures 5.40.b *Frequency grain area (mm²) histograms throughout grain development stages Buffalo primary and secondary grain.*

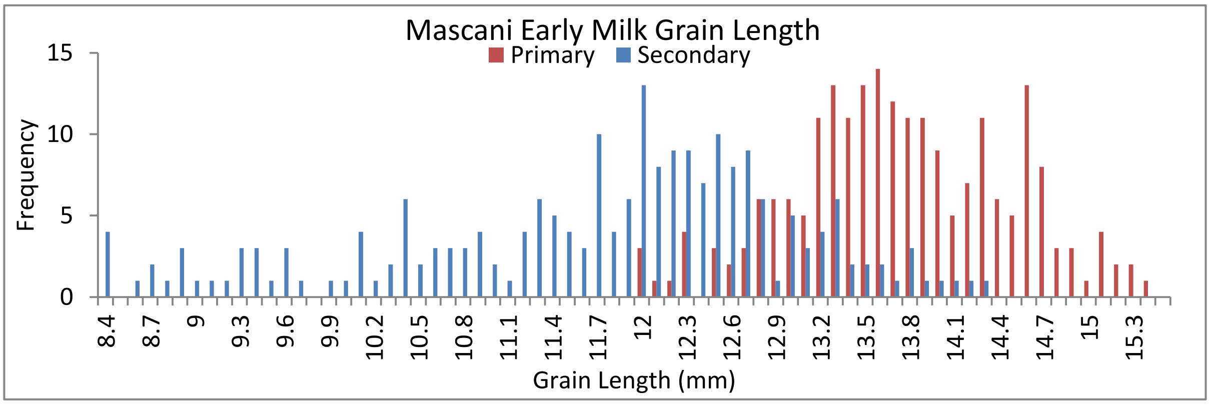


Figures 5.40.c *Frequency grain area (mm²) histograms throughout grain development stages Buffalo primary and secondary grain.*

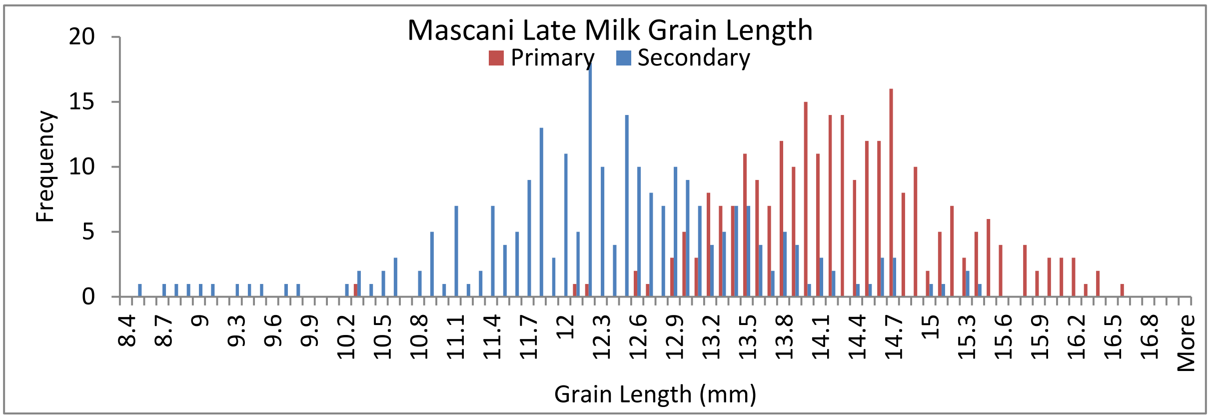


***Figures 5.40.d** Frequency grain area (mm^2) histograms throughout grain development stages Buffalo primary and secondary grain.*

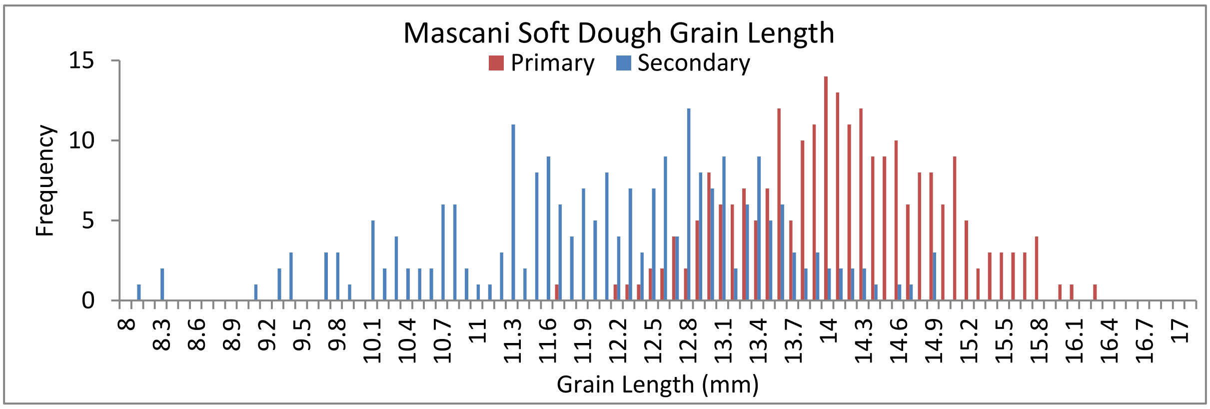
Another example of this development, figures 5.41 a to d, shows grain length of Mascani primary and secondary grain. A bimodal distribution was found from early milk although the overlap between primary and secondary grain was less evident, graphically, when compared to grain area. A reduction in the dispersion of the data when looking at frequency distribution graphs was only found at late milk. Buffalo and Tardis grain length frequency distribution histograms displayed the same performances although with different ranges of grain length (see Appendix).



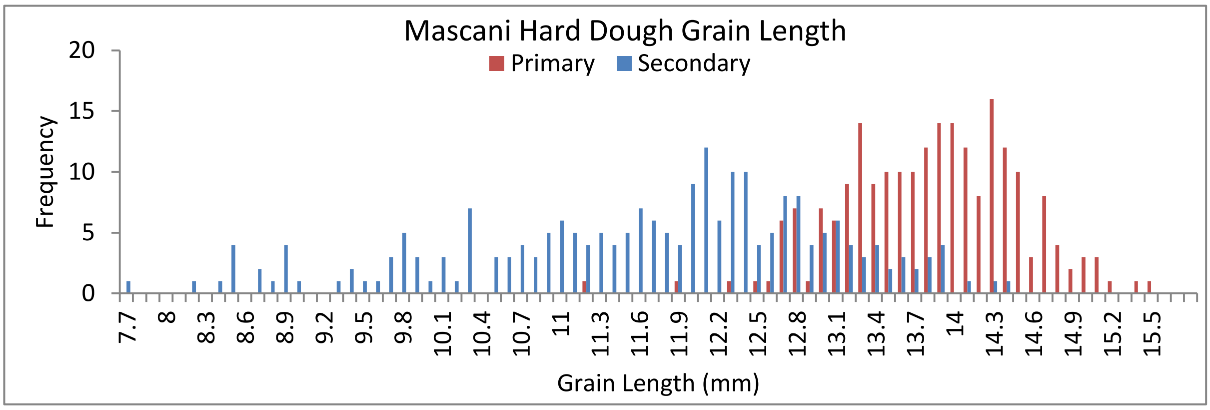
***Figures 5.41.a** Frequency grain length (mm) histograms throughout grain development stages Mascani primary and secondary grain*



Figures 5.41.b *Frequency grain length (mm) histograms throughout grain development stages of Mascani primary and secondary grain*

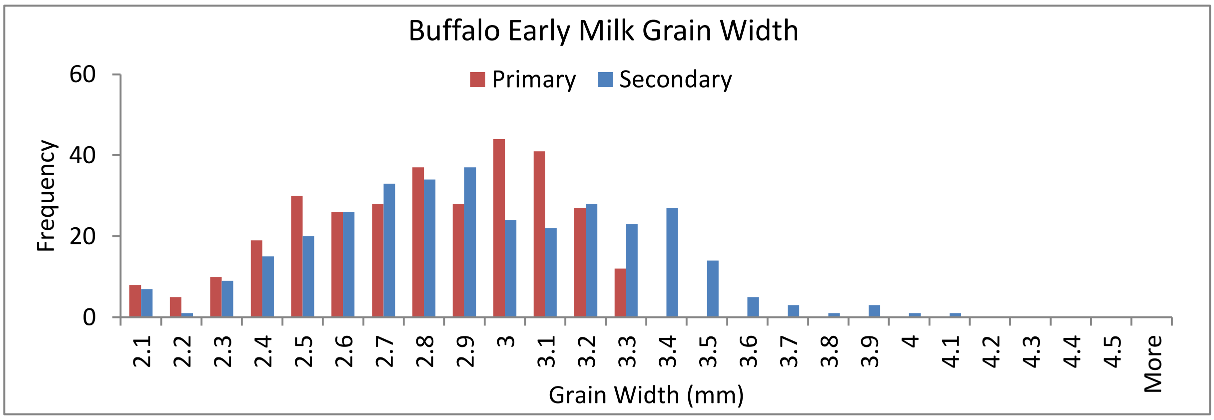


Figures 5.41.c *Frequency grain length (mm) histograms throughout grain development stages of Mascani primary and secondary grain*

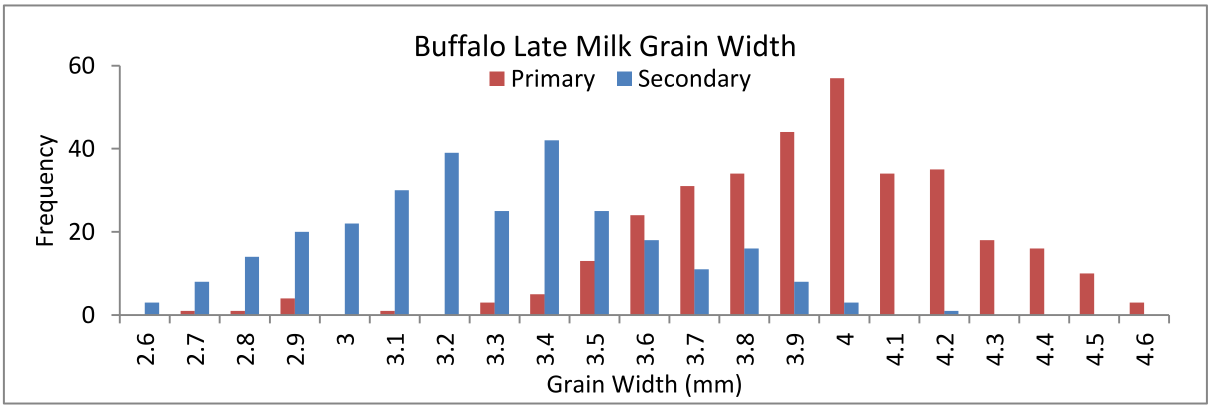


Figures 5.41.d *Frequency grain length (mm) histograms along growth development stages of Mascani primary and secondary grain.*

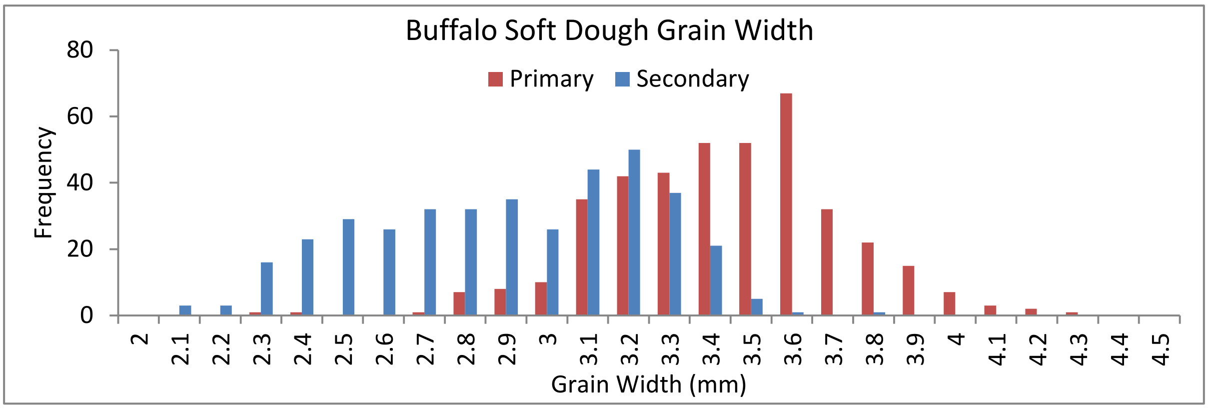
Grain width also displayed differences of development between varieties. Thus, figures 5.42.a to d, show grain width of Buffalo primary and secondary grain. Bimodality distributions were not found at early milk or hard dough, with both grain development stages showing high overlaps between primary and secondary grain. The range of grain width values was higher at late milk with maximum values of 4 mm for primary grain width. Differences between primary and secondary grain width later decreased to 3.6 and 3.7 mm for primary grain width with lower differences in comparison with secondary grain width values.



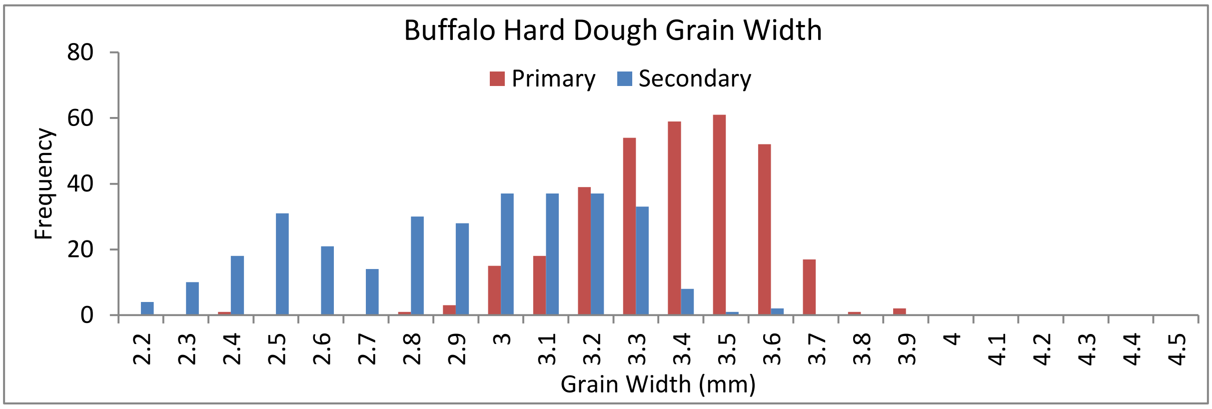
Figures 5.42.a *Frequency grain width (mm) histograms throughout grain development stages of Buffalo primary and secondary grain.*



Figures 5.42.b *Frequency grain width (mm) histograms throughout grain development stages of Buffalo primary and secondary grain*

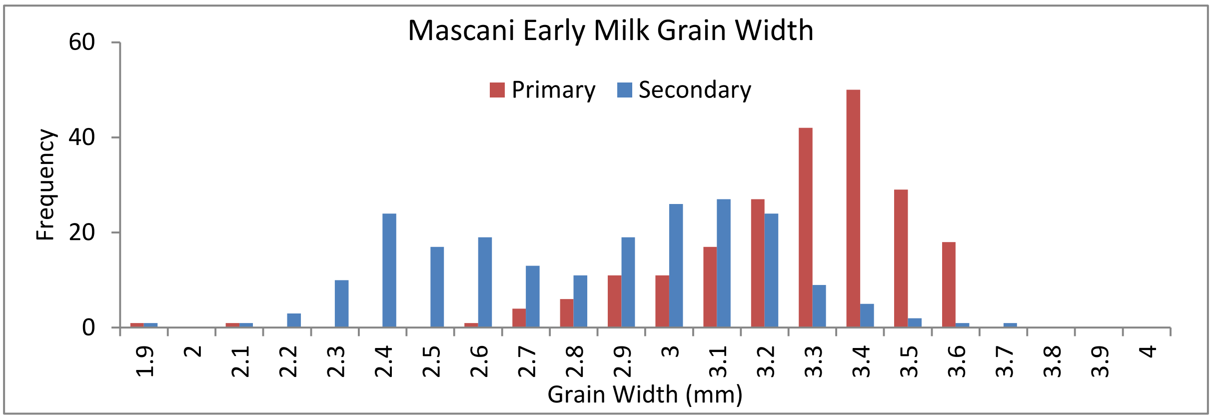


***Figures 5.42.c** Frequency grain width (mm) histograms throughout grain development stages of Buffalo primary and secondary grain*

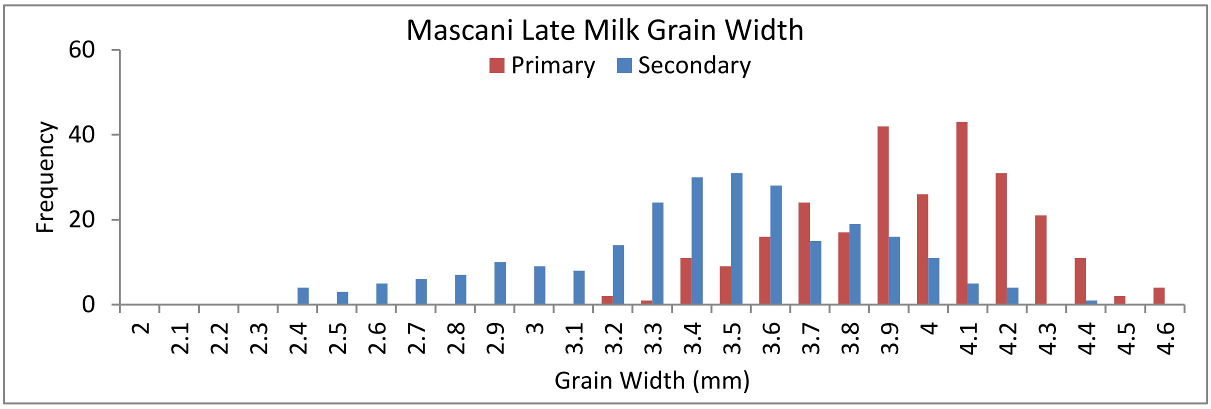


***Figures 5.42.d** Frequency grain width (mm) histograms throughout grain development stages of Buffalo primary and secondary grain.*

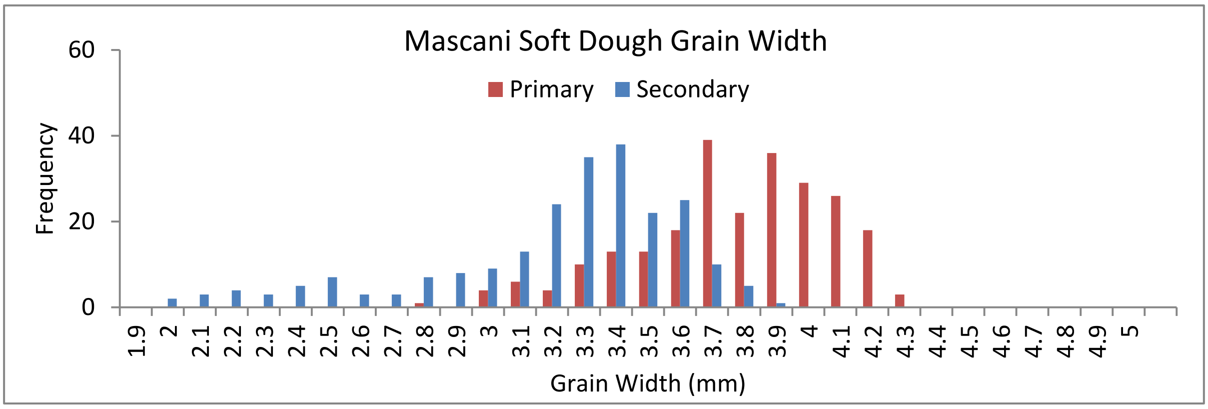
Mascani grain width (mm) (figures 5.43. a to d) showed that early milk and late milk were similar graphically, with a range of values slightly greater for primary grain width at late milk in comparison with early milk. Interestingly, a third subpopulation could be found only at early milk and the differences between primary and secondary grain width were greater. The range grain width was higher at late milk with maximum values of 4.1 mm for primary grain whilst at hard dough these values were 3.5 mm for primary grain and 3.2 mm for secondary grain width. A reduction in the dispersion of the data was found at hard dough (figure 5.43.d).



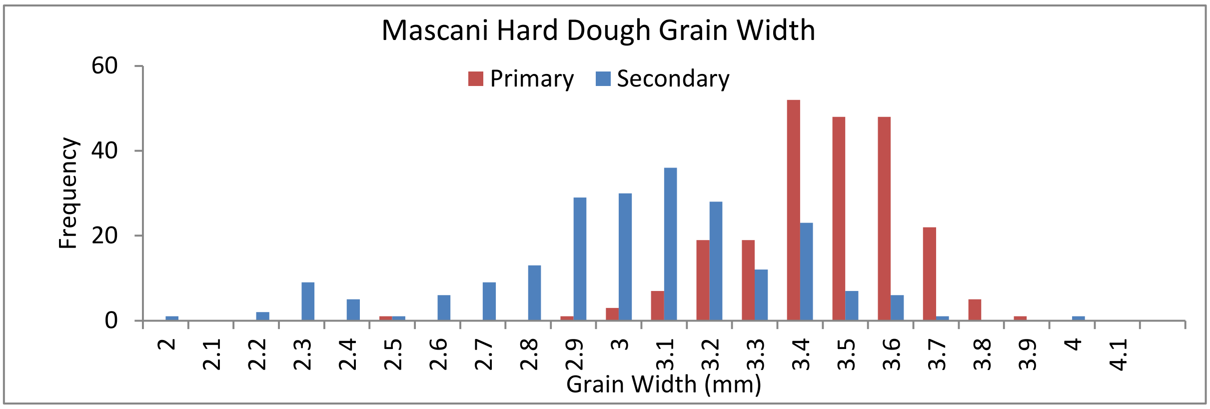
Figures 5.43.a *Frequency grain width (mm) histograms throughout grain development stages of Mascani primary and secondary grain.*



Figures 5.43.b *Frequency grain width (mm) histograms throughout grain development stages of Mascani primary and secondary grain.*

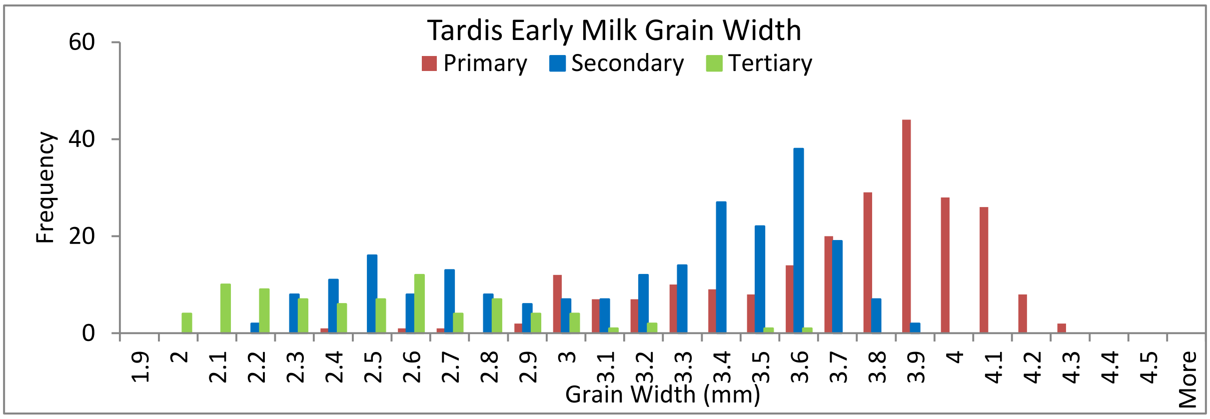


Figures 5.43.c *Frequency grain width (mm) histograms throughout grain development stages of Mascani primary and secondary grain*

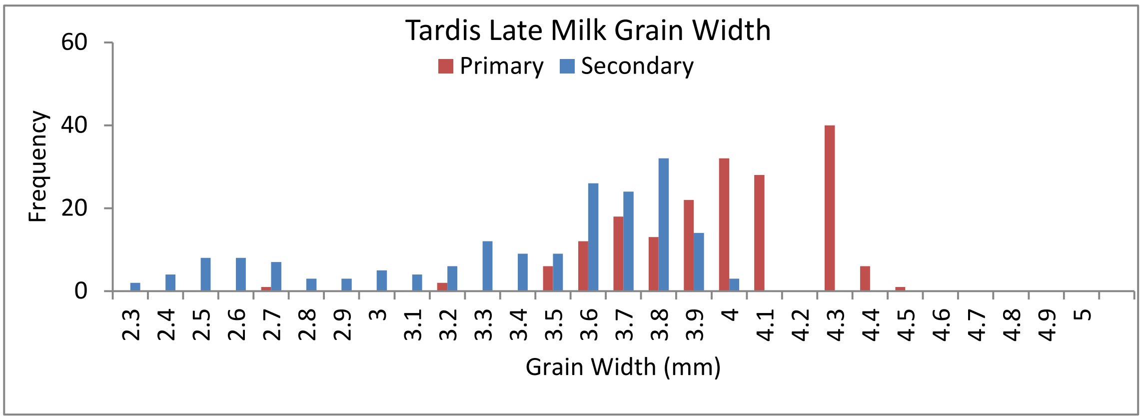


***Figures 5.43.d** Frequency grain width (mm) histograms throughout grain development stages of Mascani primary and secondary grain*

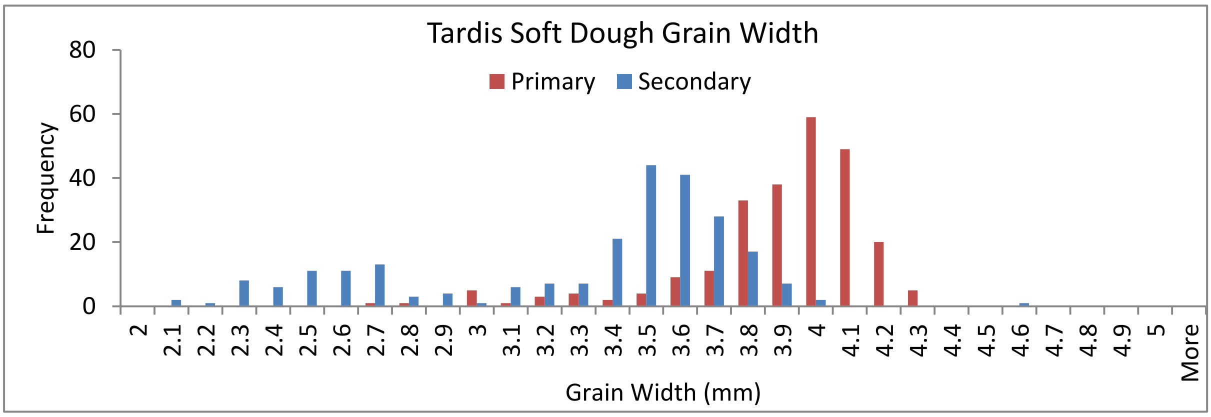
Tardis primary and secondary grain width (figures 5.44.a to d) frequency distributions histograms did not show distinct bimodality distributions except at soft dough (figure 5.44.c). The range in grain width was higher at early milk and at the same time tertiary grain was also found. A reduction on the dispersion of the data was evident at hard dough (figure 5.44.d) when the differences between primary and secondary grain width values were lower than at earlier growth stages, along with lower grain width values.



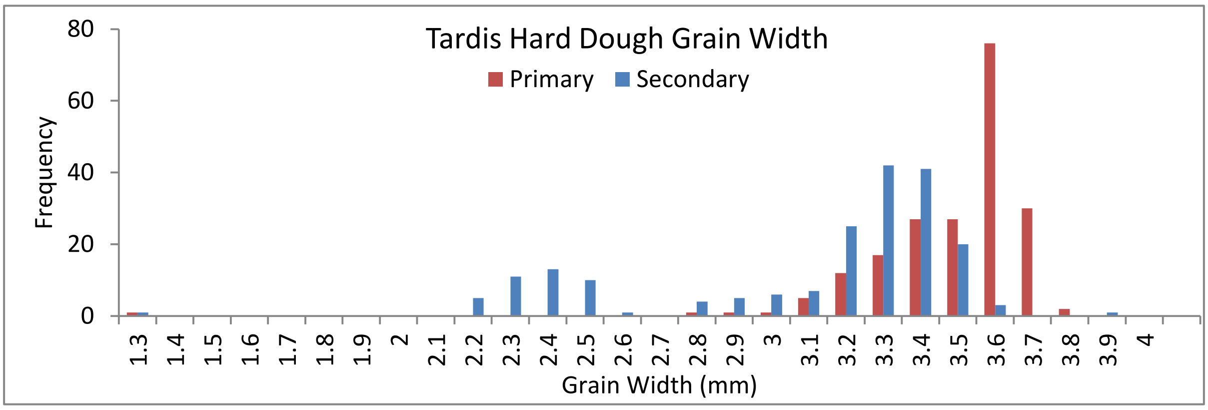
***Figures 5.44.a** Frequency grain width (mm) histograms throughout grain development stages of Tardis primary and secondary grain.*



Figures 5.44.b *Frequency grain width (mm) histograms throughout grain development stages of Tardis primary and secondary grain.*



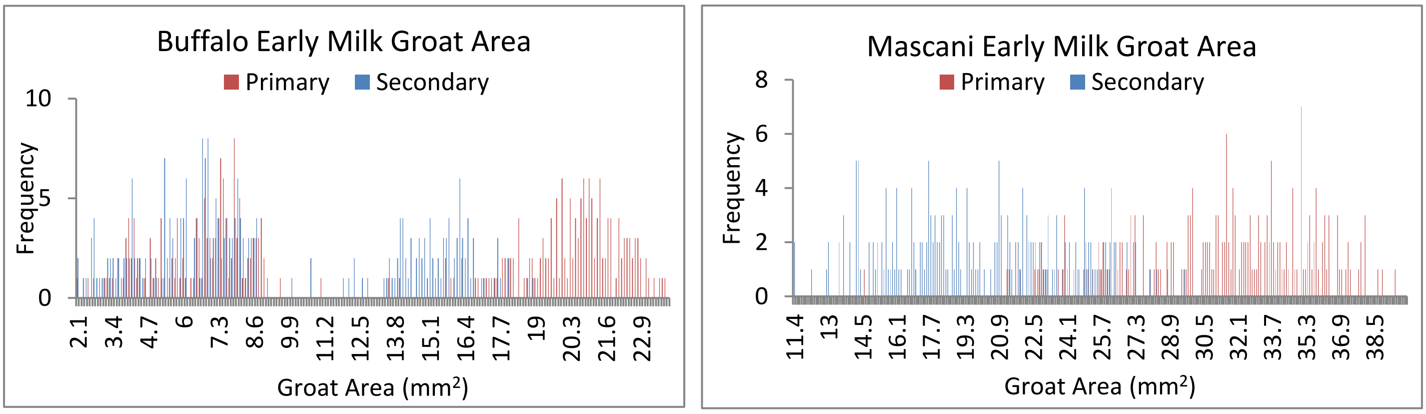
Figures 5.44.c *Frequency grain width (mm) histograms throughout grain development stages of Tardis primary and secondary grain.*



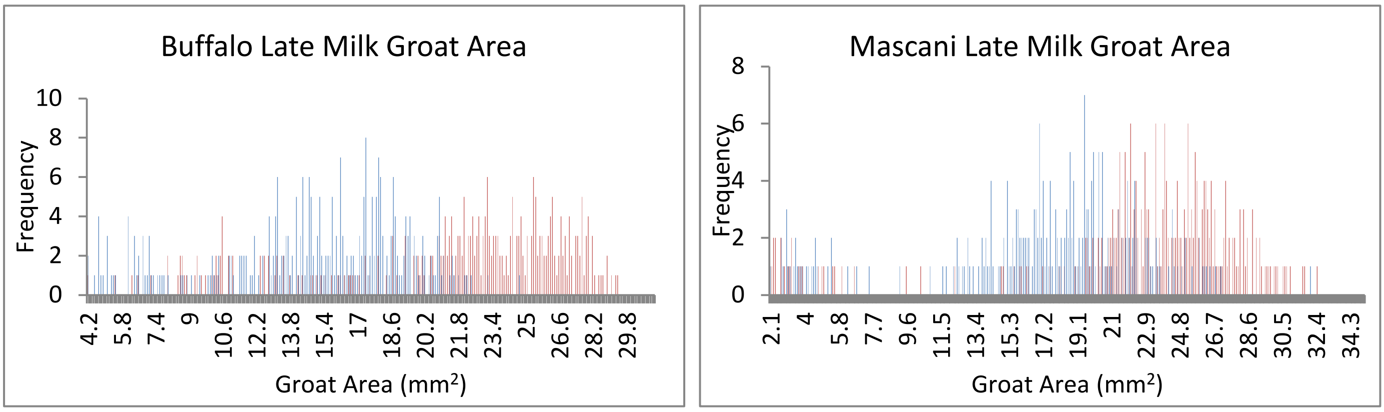
Figures 5.44.d *Frequency grain width (mm) histograms throughout grain development stages of Tardis primary and secondary grain.*

5.3.5.2 Groat size

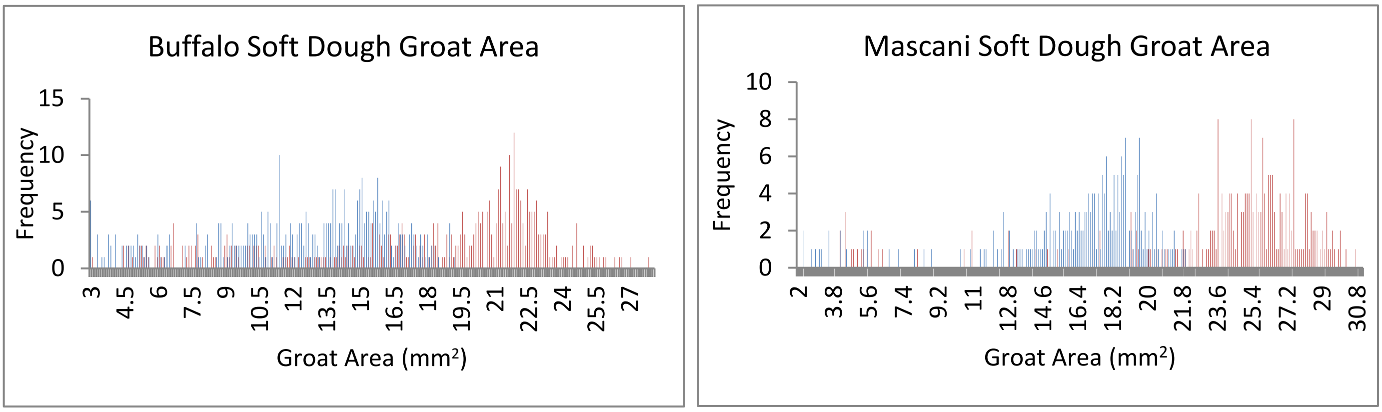
Comparisons between Buffalo and Mascani groat area (mm^2) throughout groat development allowed a comparison of the differences between the ranges of values and bimodality distributions obtained (figure 5.45 a to d). Buffalo groat areas were less than 30 mm^2 at every stage, whilst Mascani at early milk and late milk displayed higher values. Mascani groat area at early milk suggests the absence of small groats when compared to Buffalo and to other growth stages. However, at hard dough both varieties showed similar values and bimodality distributions, although the number of grains was greater for Buffalo. A number of peaks were found for primary and secondary grain particularly at the early milk stage. These represent the different size distributions found across the panicle as indicated in the mean groat area values presented above (figures 5.40, 5.41 and 5.42).



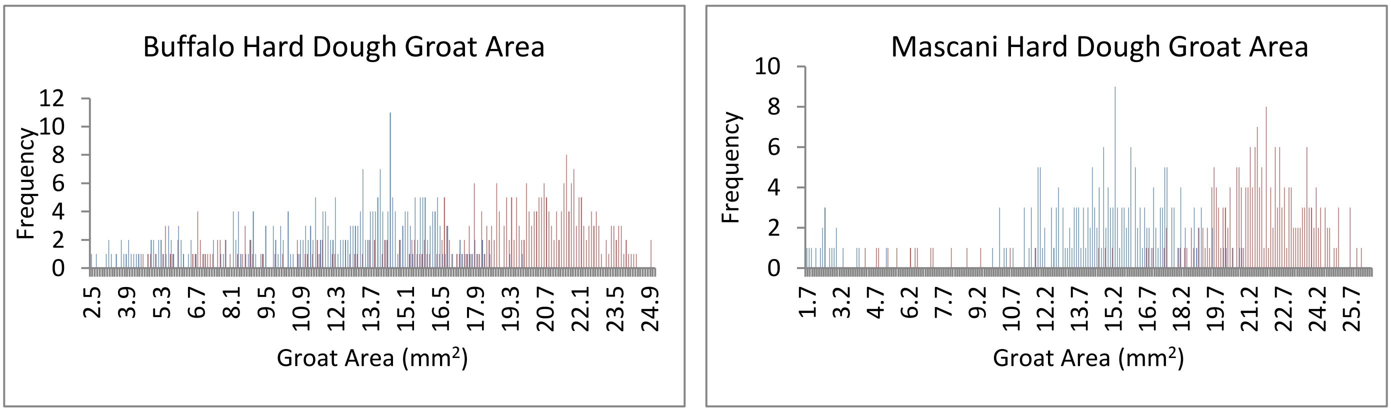
***Figures 5.45.a** Frequency of groat area (mm^2) histograms between growth development stages of Buffalo and Mascani primary and secondary grain.*



***Figures 5.45.b** Frequency of groat area (mm^2) histograms between growth development stages of Buffalo and Mascani primary and secondary groat.*



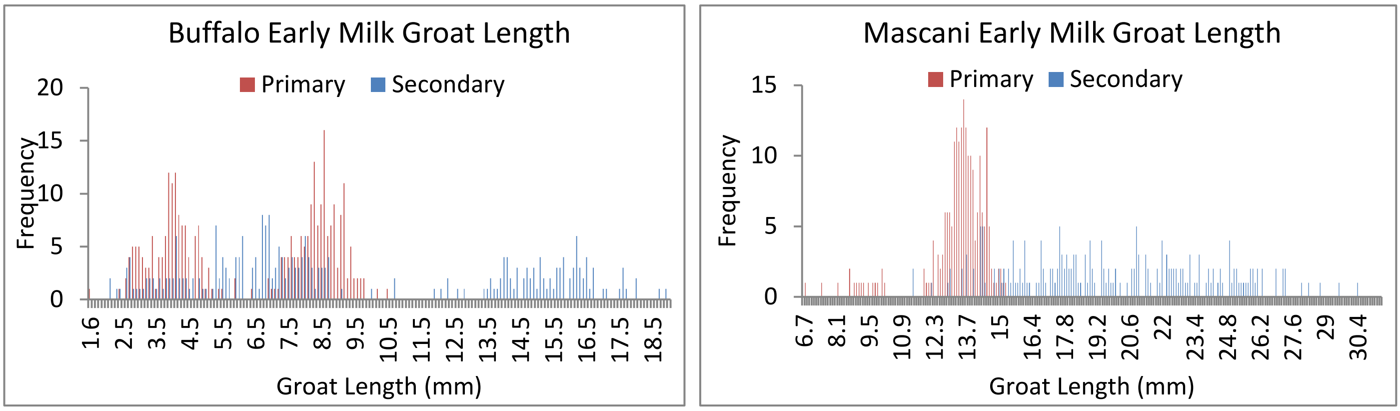
***Figures 5.45.c** Frequency of groat area (mm^2) histograms between growth development stages of Buffalo and Mascani primary and secondary groat.*



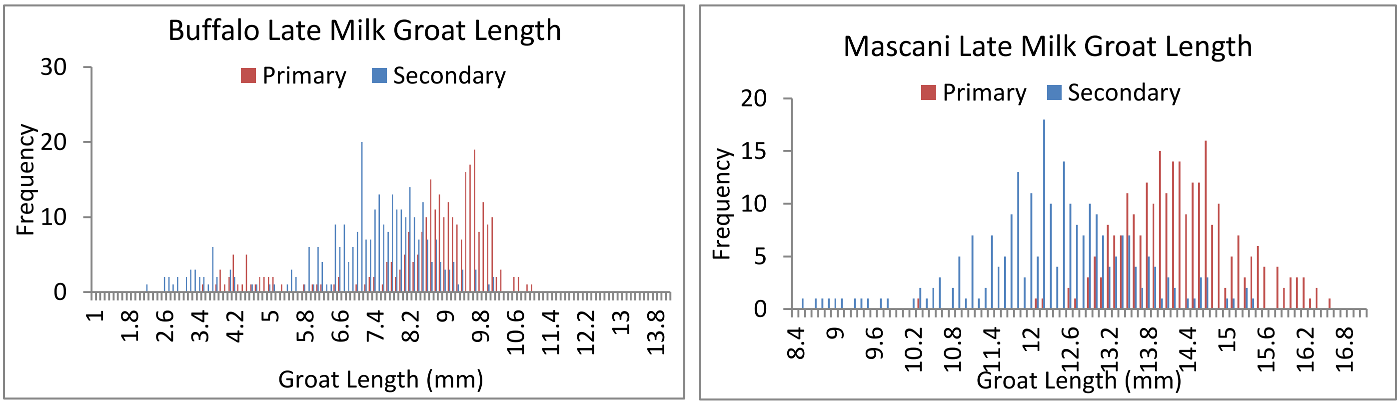
***Figures 5.45.d** Frequency of groat area (mm^2) histograms between growth development stages of Buffalo and Mascani primary and secondary groat.*

Similar patterns were found for Tardis primary and secondary groat area when compared to Buffalo (see Appendix).

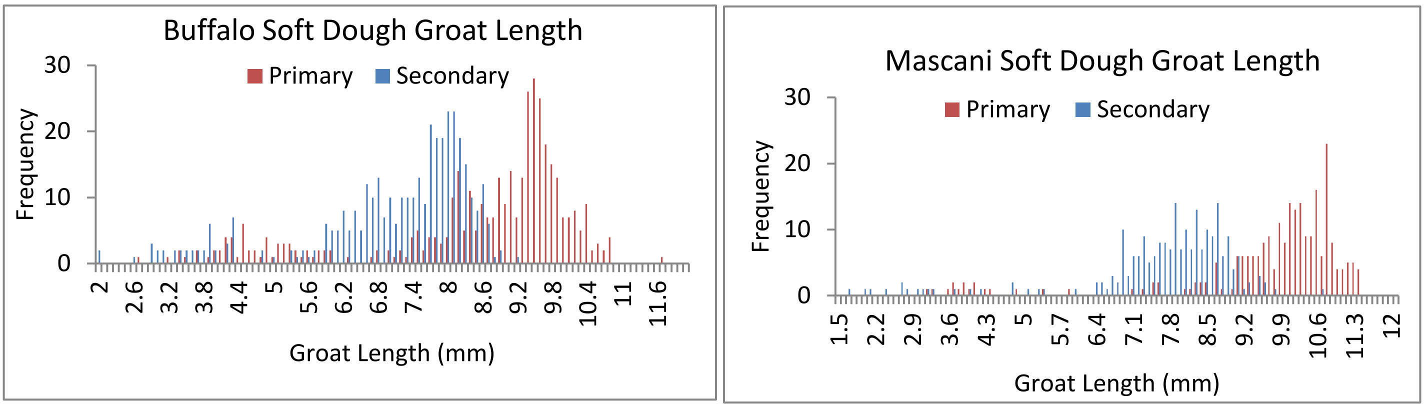
A comparison of Buffalo and Mascani groat length (mm) frequency distribution histograms throughout groat development is shown in figure 5.46 a to d, (for Tardis see appendix). As for groat area, a number of peaks were found for both primary and secondary groat length at the early milk stage with a much clearer bimodal distribution apparent at later stages. The longest groats appeared at late milk for all varieties, Mascani 14.7 mm (figure 5.46.b), Buffalo 9.6 mm (figure 5.46.b) and Tardis 10.7 mm (see appendix). The range of values decreased in all varieties and in both primary and secondary grain at hard dough.



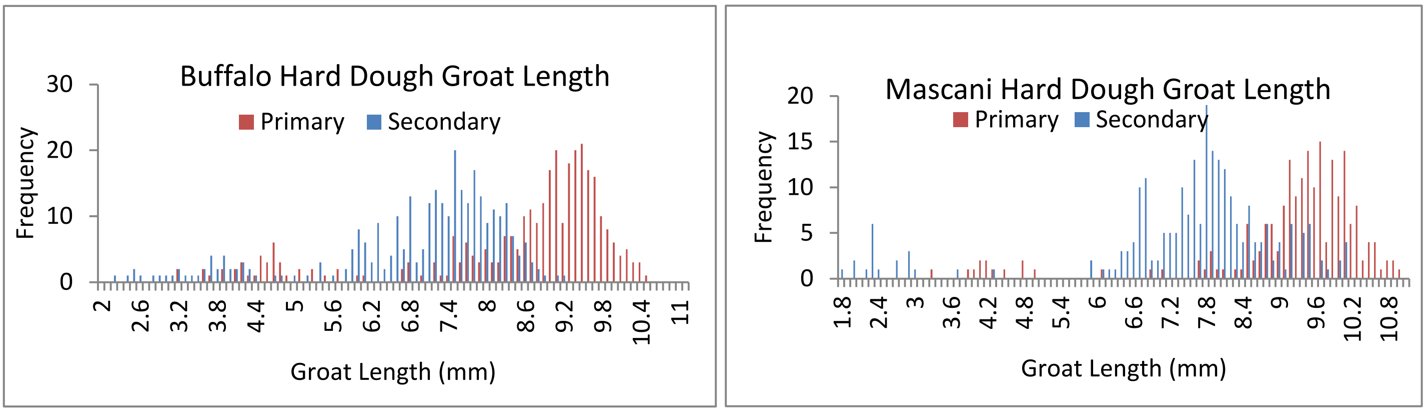
Figures 5.46.a *Frequency of groat length (mm) histograms between growth development stages of Buffalo and Mascani primary and secondary groat.*



Figures 5.46.b *Frequency of groat length (mm) histograms between growth development stages of Buffalo and Mascani primary and secondary groat.*

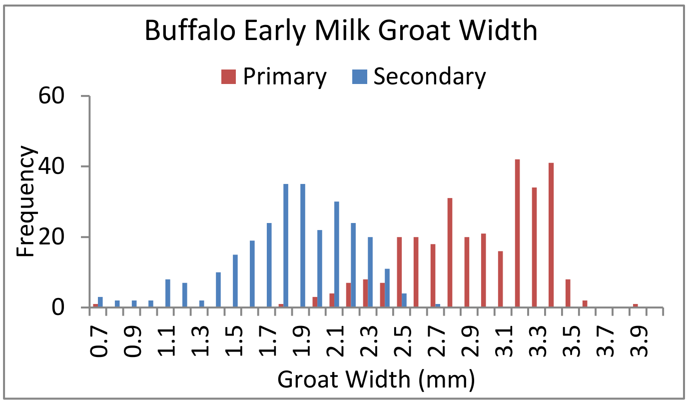


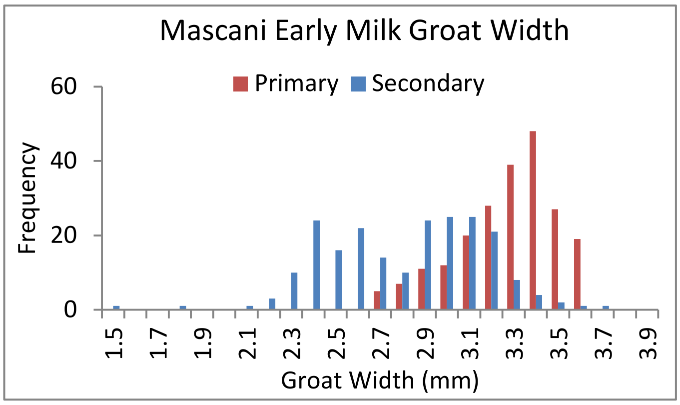
Figures 5.46.c *Frequency of groat length (mm) histograms between growth development stages of Buffalo and Mascani primary and secondary groat*



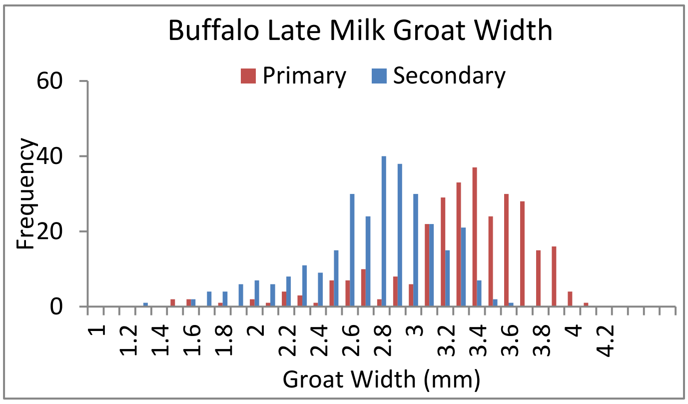
Figures 5.46.d *Frequency of groat length (mm) histograms between growth development stages of Buffalo and Mascani primary and secondary groat.*

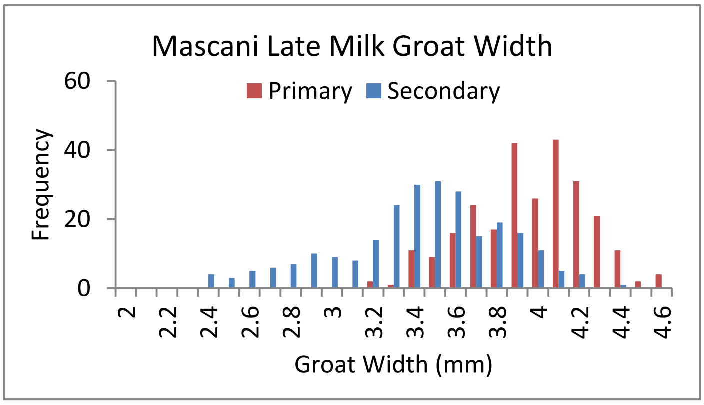
Buffalo and Mascani groat width (mm) and Tardis (see Appendix) frequency distribution histograms throughout groat development displayed a progressive loss of bimodality distribution. The ranges in groat width values (figure 5.47 a to d) for all varieties and for both primary and secondary groat also decreased during the development. Thus, at early stages thinner groats were found with minimum values of 1.1 mm for Buffalo and Tardis whilst Mascani showed minimum values of 1.4 mm width. At hard dough the overlap between the two subpopulations, primary and secondary groat increased so that the bimodality was lost by hard dough.



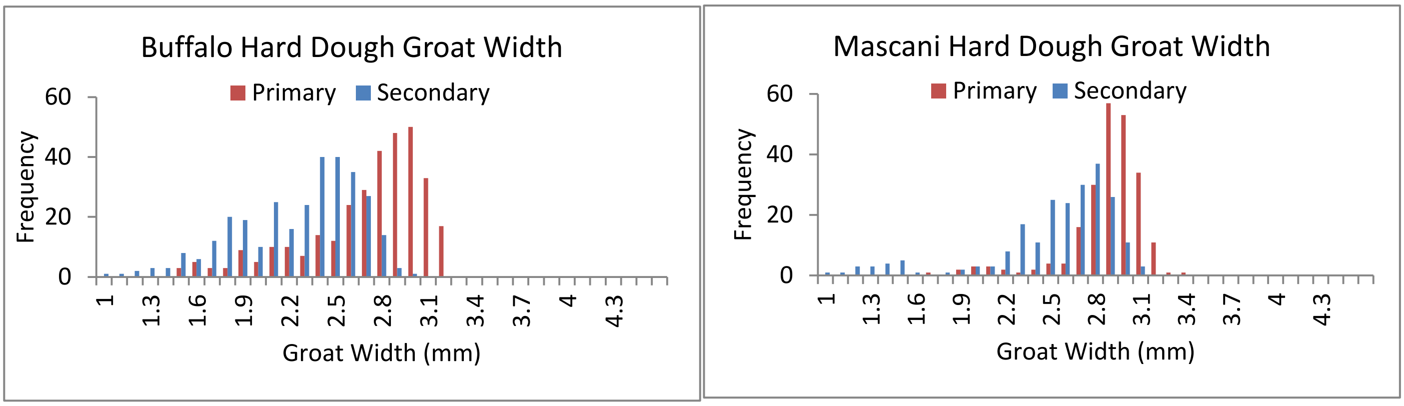


Figures 5.47.a *Frequency of groat width (mm) histograms between growth development stages of Buffalo and Mascani primary and secondary groat.*

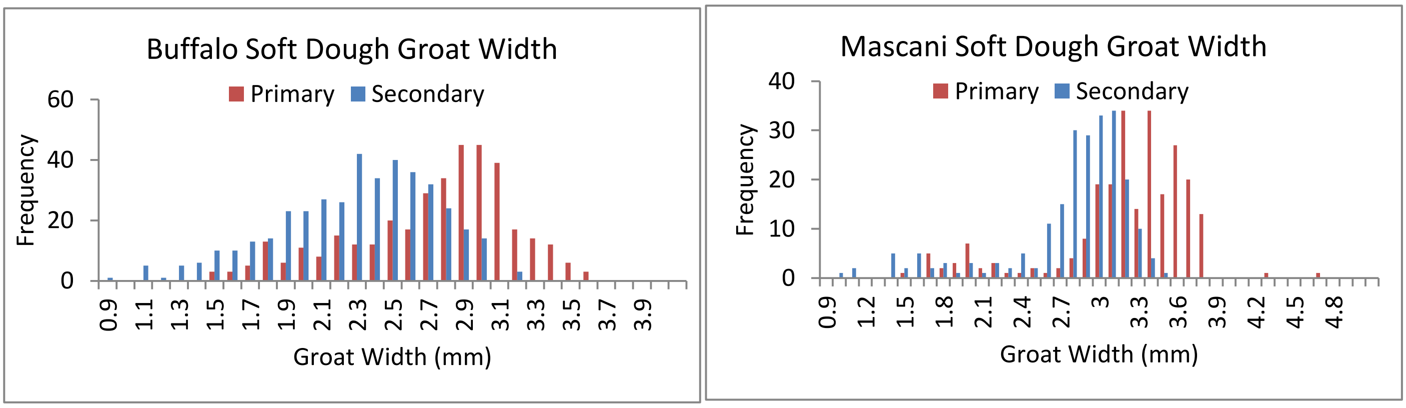




Figures 5.47.b *Frequency of groat width (mm) histograms between growth development stages of Buffalo and Mascani primary and secondary groat.*



Figures 5.47.c *Frequency of groat width (mm) histograms between growth development stages of Buffalo and Mascani primary and secondary groat.*



Figures 5.47.d *Frequency of groat width (mm) histograms between growth development stages of Buffalo and Mascani primary and secondary groat.*

5.4. Discussion

Developing oat varieties with high milling quality traits and at the same time high yield is the focus of researchers and breeders to make oats a competitive cereal against other crops. Despite its economic importance, there is little knowledge of the physical and genetic factors influencing and affecting quality in oats.

Few studies so far, have looked at how development in oat panicle and grains affect grain quality parameters of importance for the milling industry and end-users. Agro-ecological factors have been previously reported, e.g. drought, fertilization levels, type of soil, etc. as influencing yield (Browne et al,, 2006). Other grain quality parameters, such as specific weight, kernel content, hullability, and chemical quality parameters, have been studied under different management conditions and environments, with diverse results being found on the importance of each factor on final grain quality (Ohm, 1976; Marshall & Murphy, 1981; Givens et al., 2004).

There is currently no oat specific grain development guidance. This study provides a physical and comparative analysis on grain and panicle development identifying possible quantitative reference points against which the best management conditions can be adjusted to oat performance according to panicle and grain peculiarities.

The oat inflorescence consists in a panicle which bears whorls, which at the same time bear branches where we find the spikelet. Inside each spikelet two to three grains called primary, secondary and tertiary grain, which are protected by a leaf like structure, the husk. The characteristics of the panicle influence the variability in grain size and shape between types of grain, and therefore might affect grain quality parameters. It has been suggested that grain quality depends on the development of individual grains and that season, site and variety rather than management conditions, are crucial factors affecting development and growth and therefore grain quality (Browne et al., 2006).

How the panicle, grain and groat develop and influence grain quality was studied in detail at the present chapter during two seasons, (2014/2015 and 2015/2016) in the field using three varieties under standard management conditions. The first season was designed as preliminary experiment to set the basis of sampling timing for the second and whilst in the first one only five points of grain development, i.e. early milk, late milk, soft dough, hard dough and ripening were selected, changes observed in grain development suggested more sampling points in between growth stages (data non-shown).

In both seasons each variety under study showed a unique pattern of development for each trait studied i.e. kernel content, thousand grain weight, moisture content and grain and groat size parameters. However, some similar of grain and groat development points were also found.

In general, kernel content increased during grain development, with higher values at the top of the panicle than the rest of whorls. Secondary grain had higher kernel content than primary grain throughout grain development with lower differences at final stages. Kernel content was more similar pattern between Buffalo and Tardis but irregular in Mascani. Buffalo and Tardis secondary grain had higher kernel content than the primary grain at the hard dough stage whilst Mascani secondary grain developed kernel content differently so meaning that despite having values above primary grain from early stages of development, at hard dough kernel content in secondary grain was lower than in the primary grain.

Thousand grain weight during development showed significant differences between varieties, primary and secondary grain, growth stages and interactions between them. However, similarities were also found between Buffalo and Tardis which reached maximum values at late milk. Secondary grain thousand grain weight always lower than that of the primary grain. Mascani, on the other hand, showed a different pattern, with maximum values at soft dough and secondary grain thousand grain weight values at hard dough similar to those of primary grain.

Despite the variety differences described above, there were also important common points in the development of all traits studied. Primary and secondary grain displayed the same pattern within varieties. At the same time, there were differences between the top and the bottom of the panicle, i.e. whorls, for all grain quality traits and grain and groat size and shape. Underlying this difference across the panicles is the wide range in flowering time observed in oats with the top florets flowering much earlier than those at the base of the panicle. Grain developing at the top of the panicle thus has a much longer time between flowering time and eventual harvest as compared to those developing at the base of the panicle.

Moisture content was similar throughout grain development for all varieties. The top of the panicle had lower moisture contents that progressively decreased until reaching a minimum at hard dough. Secondary grain had higher levels of moisture at early stages of development in all varieties and interestingly, at hard dough, which might explain a tighter husk affecting hullability of this grain (Browne et al., 2002).Tardis and Buffalo had lower moisture content than Mascani. All varieties had values similar to that required in the market at harvest, approximately 14%, to avoid storage problems.

Grain size and shape showed statistically significant differences between varieties, type of grain and growth stages and also the interactions between them were significant. Grain area showed less variability during development in all varieties with similar values from early milk until hard dough and the same patterns between the top and the bottom and between primary and secondary grain. Rates of change between different stages were higher between secondary grain and at the bottom of the panicle when compared to primary grain at the top of the panicle.

Development of grain length was different between varieties. Buffalo and Tardis reached maximum values at soft dough whilst in Mascani the longest grains were found at hard dough. However, only Buffalo increased grain length during grain development. Secondary grain for all varieties had higher rates of change than primary grain

Grain width was statistically significantly different for varieties, type of grain and growth stages and significant interactions between variety and growth stage and between types of grain. In general, for all varieties less variability was found than in grain length, with smaller differences between early milk, late milk and hard dough grain. Maximum values were found at soft dough for Buffalo and late milk for Mascani and Tardis. It is important to point that higher whorls and the top of the panicle reached maximum values before lower whorls in all varieties. Rates of change were similar values between primary and secondary grain within varieties at the same growth stage.

Differences were not found for any of the varieties when looking at groat sizes, including area, length and width. All varieties reached maximum values for groat dimensions at late milk, with groat sizes at the top of the panicle being higher than the rest of the panicle. Groat width showed greater reductions from late milk until hard dough than groat length in all varieties.

Frequency distribution histograms analysis showed a reduction in the range of values as development progressed, i.e. a reduction in the dispersion of the data. The overlap between primary and secondary grain area and length increased for all varieties as development progresses but a bimodal distribution due to the differences between the two types of grain was always apparent. Interestingly grain width only displayed a clear bimodality distribution at early stages of grain development. The presence of tertiary grain was not detected for all stages and varieties despite being previously reported to be found particularly in Tardis (Howarth pers.Com). It has been suggested that this is due to an over-abundance of photosynthate (Doehlert, McMullen & Riveland, 2002), but in this study was only found in certain plants rather than being a constant across the field.

The results found for grain and groat size suggest that width is a grain size trait reaching maximum values before grain length during in grain development, which might be the determinant on final grain area. The differences in development in all traits between primary and secondary grain might explain the differences previously reported in quality traits and in grain and groat size between the two types of grain. Primary grain is usually described as longer, with higher thousand grain weight, lower kernel content and poorer hullability (Tibelius & Klinck, 1986; Browne et al., 2002). The decreased in groat width coincides with the highest drop in moisture content previously mentioned, supporting the idea that the shrinking effect on the seed with the loss of moisture allows the hull to detach from the groat. At a physiological level this might allow suggesting that width is the main grain plastic trait in moisture content inside the husk. At the same time it might be argued that as the groat develops inside the husk, the kernel would reach first maximum groat width values, then grows in length. Therefore, as the groat matures and loses moisture content this mainly affects groat width in secondary groats supporting the higher hullability previously reported for secondary grain (Browne et al., 2002). This could have important implications on hullability, facilitating the separation between the husk and the groat in the dehulling process. It would be interesting to analyse in the future changes and effects of moisture content on hullability throughout grain development.

At developmental level, as previously reported in wheat and rice (Gegas, Nazari, Griffiths, Simmonds, Fish, Orford, Sayers, Doonan, & Snape, 2010) the independent development found between width and length may reflect differential modulation in growth that can be titled as growth arrest along the main stem of the panicle and along grain axes, at different developmental stages. It would be very interesting to conduct further analysis of the data produced in this chapter to develop a growth model for grain development across the panicle in oats and determine the optimal time for harvest so that grain quality traits are maximised.

5.5 Conclusions

- Kernel content increased during grain development with higher values at the top of the panicle, but no significantly statistically different within primary and secondary grain within any variety at any growth stage.
- Thousand grain weight during development showed significant differences between varieties, primary and secondary grain, growth stages and interactions between them. Secondary grain thousand grain weight always lower than that of the primary grain.
- Moisture content was similar throughout grain development for all varieties. The top of the panicle had lower moisture contents that progressively decreased until reaching a minimum at hard dough. Secondary grain had higher levels of moisture at early stages of development.
- Buffalo and Tardis showed similar kernel content, thousand grain weight and moisture content patterns but irregular in Mascani. Secondary grain in all varieties had values above primary grain from early stages of development, till hard dough when reaching similar values.
- The results found for grain and groat size suggest that width is a grain size trait reaching maximum values before grain length during in grain development, which might be the determinant on final grain area. This growth arrest might reflect differential in modulation in growth along the panicle and throughout grain development.

Chapter six. General discussion

The last few decades have seen an increasing scientific discussion on grain quality in oats and its sustainability in a more efficient production system. However, the oat production system is different from wheat and barley due to its status as a relatively minor crop, with oats ranking seventh within cereals grown worldwide. Therefore, the scope for research and the posterior impact in the market is significantly reduced when compared with wheat and barley. Oat breeders, following the market, end-users and farmers´ preferences, focus on developing oats with yield as well as with grain quality traits. Developing new oat varieties requires a comprehensive and better understanding of the impact of crop management, physical, environmental and genetic factors, and physiological processes, all of them potentially affecting or influencing grain quality parameters.

In this study many of the aspects above mentioned involving physical, genetic, environmental and physiological development processes were considered to unpack the basis of grain quality parameters in oats.

6.1 Genotype by environment analysis

In chapter one, one main experiment was designed and developed, involving two harvest seasons and four winter oat varieties grown at major environments of crop production across the UK. The aim was to characterize the environmental by genotype influence on grain quality parameters. Several milling quality traits and grain size and shape were measured and a range of statistical analyses conducted. Several statistical analyses were applied to the data, including Pearson's correlation, joint regression analysis (Finlay & Wilkinson, 1963) and non-parametric stability coefficients (Becker & Leon, 1988).

Results showed that none of the varieties displayed a superior performance in all quality traits nor did any one site showed a superior performance over all values for all varieties. Yield (t/ha) over seasons, was significantly different across environments but not between varieties, showing a higher influence of environmental factors than genotype, whilst specific weight (kg/hl), was significantly different between environments and varieties, although without interaction between them indicating both genotype and environmental influence. Kernel content (%), hullability (%) and thousand grain weight showed variable results. It would be interesting to look in detail at the grain retained during dehulling, to determine what features might affect the differences found in kernel content and hullability between varieties and sites.

Chemical traits displayed an interesting mirror effect due to a negative correlation between protein and oil content meaning that those varieties with high protein content showed low oil content. Although these results do not allow the establishment of a causal relationship between the two parameters, they indicate that environmental conditions that result in high protein content are associated with low oil content and therefore more suitable for human consumption.

A comparison of all the joint regression and stability parameters analysis (table 6.1.a and 6.1.b) for all varieties and quality parameters is given to assist in summing up all the results obtained.

Mascani was, according to all statistical analysis and coefficients calculated (table 6.1.a and 6.1b), the most stable variety, with the lowest sensitivity to the environment. Mascani also had the highest values of kernel content, hullability and thousand grain weight and grain width, and groat area and width, although these two last two groat size traits were not constant for all stability coefficients calculated. Gerald was superior in stability coefficients and sensitivity values calculated for specific weight although with the lowest mean values for β-glucan and grain length. Tardis was superior to the other three varieties in terms of mean oil content, although the most stable values were found for Gerald for oil and protein content giving predictable responses.

Chemical results suggest that Throws farm in 2014 was the site the highest overall mean oil and protein contents not β-glucan content. The interactions between environment and genotype suggested that niche-matching varieties according to the chemical quality trait of interest could be conducted.

Environments and seasons where the varieties were grown displayed variable grain quality results. Sites where removing the environment effect showed higher differences between varieties are more suitable to future further investigations on analysing grain quality differences in terms of genotype by environment interactions.

Table 6.1.a *Summary of stability coefficients and sensitivity values of each variety and parameter from chapter one, genetic by environment factors influencing grain quality parameters. Number refers to the rank order of each parameter calculated for the 4 varieties tested. *n.s. =non-significant.*

Varieties	*Yield*					*Kernel Content*				
	means	Superiority	Stability	Sensitivity	Mean square	means	Superiority	Stability	Sensitivity	Mean square
Balado	n.s.	2	4	4	4	4	4	4	2	4
Gerald	n.s.	1	2	2	1	2	2	2	4	3
Mascani	n.s.	4	3	3	3	1	1	1	1	1
Tardis	n.s.	3	1	1	2	3	3	3	3	2

	Specific Weight					*Hullability*				
	means	Superiority	Stability	Sensitivity	Mean square	means	Superiority	Stability	Sensitivity	Mean square
Balado	4	4	4	n.s.	4	3	3	4	4	3
Gerald	1	1	1	n.s.	1	2	2	2	2	2
Mascani	2	2	2	n.s.	2	1	1	1	1	1
Tardis	3	3	3	n.s.	3	3	4	3	3	4

	Thousand Grain Weight					Oil				
	means	Superiority	Stability	Sensitivity	Mean square	means	Superiority	Stability	Sensitivity	Mean square
Balado	2	3	4	4	3	2	2	4	4	4
Gerald	4	4	2	3	1	3	3	1	1	1
Mascani	1	1	1	1	4	4	4	3	3	3
Tardis	3	2	3	2	2	1	1	2	2	2

	Protein					*Beta Glucan*				
	means	Superiority	Stability	Sensitivity	Mean square	means	Superiority	Stability	Sensitivity	Mean square
Balado	1	1	4	4	2	1	1	2	2	3
Gerald	3	3	2	2	1	4	4	1	1	1
Mascani	3	4	3	3	3	2	2	3	3	4
Tardis	2	2	1	1	4	3	3	4	4	2

Table 6.1.b *Summary of stability coefficients and sensitivity values of each variety and parameter from chapter one, genetic by environment factors influencing grain quality parameters. Number refers to the rank order of each parameter calculated for the 4 varieties tested*

Varieties	*Grain area*					*Grain width*					*Grain length*				
	means	Superiority	Stability	Sensitivity	Mean square	means	Superiority	Stability	Sensitivity	Mean square	means	Superiority	Stability	Sensitivity	Mean square
Balado	1	2	4	4	2	3	3	4	4	4	2	2	3	3	4
Gerald	4	4	3	3	1	4	4	2	3	2	4	4	1	1	1
Mascani	3	3	2	2	4	1	1	1	1	1	2	3	4	4	2
Tardis	2	1	1	1	3	2	2	3	2	3	1	1	2	2	3

	Grain ratio				
	means	Superiority	Stability	Sensitivity	Mean square
Balado	3	3	3	3	2
Gerald	1	1	1	1	1
Mascani	2	2	4	4	4
Tardis	4	4	2	2	2

	Groat area					*Groat width*					*Groat length*				
	means	Superiority	Stability	Sensitivity	Mean square	means	Superiority	Stability	Sensitivity	Mean square	means	Superiority	Stability	Sensitivity	Mean square
Balado	2	3	4	4	2	4	4	4	4	3	2	2	4	4	3
Gerald	4	4	2	3	1	3	3	1	2	2	4	4	2	3	1
Mascani	1	1	1	1	2	1	1	2	1	1	1	1	3	2	4
Tardis	3	2	3	2	3	2	2	3	3	2	3	3	1	1	2

The negative and positive correlations found between grain size and shape in each of the varieties showed the relation of area (mm^2), length (mm) and width (mm) over each quality parameter. Although correlation does not imply causation, these results might allow developing prediction models. These models would take grain size and quality parameters values throughout the season to predict final yield and milling quality parameters.

Bimodality distributions and parameters were confirmed by image analysis and posterior bimodality distribution frequency calculations on grain and groat size and shape. Primary and secondary grain and groat showed bimodal distributions for all traits and varieties regarding size and shape although the most apparent was in terms of grain length. Grain and groat area showed a stronger variation for length, meaning a stronger correlation than with width, for all harvest seasons and varieties.

The overlap found between the two-subpopulations of grain sizes was variable, with different values of the proportion of individuals for the two subpopulations for all traits and varieties regarding grain and groat size and shape (Fogelfors M., Peterson B., 2004). These results might be explained by panicle development in oats. Oat spikelets comprise usually of two to three grains (Welch, 1995), with the primary one larger in comparison to the secondary and the tertiary grain (Browne et al., 2002), although the primary grain being at the same time poorer in kernel content and hullability (Browne et al., 2002). In this study, the primary and secondary grain were not analysed individually and therefore, the subpopulation under the curve in the bimodality graph and proportions calculated do not include exclusively primary or secondary grain but also a certain number of grains that could belong to one or another category, making difficult to establish the limits between them. Further development in the mathematical method to assess those parameters and exclude the odd values also found during this analysis is needed. This approach could lead to a new quality parameter due to the influence of grain and groat size on posterior processes in the milling industry (Symons & Fulcher, 1988).

The variability found between environments, years and varieties allow us to suggest that locally adapted varieties would perform better. Therefore, niche-matching varieties according to historical performance in local environments rather than overall performance of the variety would allow reaching higher grain quality parameters for end-users and milling industry requirements.

6.2 Nitrogen response of grain quality parameters.

Establishing optimum levels of nitrogen to apply as fertilizer to oats is particularly important to enhance its competitiveness among other cereals. The focus for breeders is on developing varieties with higher yield and stability that requires the minimum fertilizer to reduce environmental impact and unnecessary costs, without compromising grain quality parameters.

Three experimental trials, two at Rosemaund (ADAS) in 2014/2015 and 2015/2016 harvest seasons, and one at Gogerddan (IBERS) in 2014/2015, were developed with different treatments of nitrogen as fertilizer. The application of nitrogen has been shown to have a positive effect on yield displaying a strong significant positive correlation for all varieties and sites, with non-significant differences between varieties with increasing levels of nitrogen.

None of the milling quality traits displayed a significant linear response to nitrogen level except hullability at one site, ADAS 2014. A better association have been found when curves had been fitted both data. However a plateau was not reached at any milling quality parameter.

Specific weight was, in accordance with previously reported results (Ohm, 1976; Givens et al., 2004), lower with higher levels of nitrogen at all sites, although with variability by sites. Several factors might explain this effect. Firstly, incomplete grain filling and therefore less dense grains, due to competition because of an increased shoot number as has been previously found to be correlated with higher levels of nitrogen applied (Chalmers et al., 1998; Browne et al., 2004; Muurinen et al., 2006). Increased levels of nitrogen resulted, at the same time, in higher grain lengths but lower grain width, which might result in hulls more loosely attached to the groat and therefore reduce specific weight. Finally, positive correlations were found between specific weight and grain ratio and density. Specific weight is a measure of bulk density and is affected by both the density of the grains and how well they pack. According to the correlations found, the reductions in specific weight with nitrogen applied on specific weight might be due to greater effect of the grain ratio rather than due to the increase effect of nitrogen applied on grain density. Specific weight is used extensively to grade oat and other cereals before milling, and it is thought to be related to grain shape and size since these parameters determine the way the individual grain packs (Gegas, Nazari, Griffiths, Simmonds, Fish, Orford, Sayers, Doonan, Snape, 2010). As grain ratio increases with nitrogen applied the relation grain width and length closes to one, making the grain more uniform and therefore higher number of grains per liter bulk grain.

Incomplete grain filling due to competition because of an increased shoot number with higher levels of nitrogen, might also explain the positive effect of nitrogen on hullability, due to a more loosely attached husk to the groat. However, this hypothesis also suggests a negative effect on kernel content. However, results showed that increasing levels of nitrogen had a positive effect on kernel content. The positive correlation found between grain density and kernel content and the positive effect of increasing levels of nitrogen on grain density, might explain the positive effect on kernel content. Denser grains are heavier, increasing the weight of the groat and so kernel content.

Grain and groat area and length were positively affected by increasing levels of nitrogen. Analysis of grain and groat frequency values confirmed a bi-modal distribution for grain and groat area and length parameters as found in chapter three, representing the primary and secondary grain found in each spikelet. At the same time, increasing levels of nitrogen resulted in diminishing of the bi-modal character of those distributions. The overlap between the two sub-populations increased, having as a consequence, a homogenization of the proportion of the two sub-populations under the curve with groat area and length more affected than grain size traits.

In this chapter several positive and negative effects of increasing levels of nitrogen on grain quality parameters were found. Increasing levels of nitrogen had a negative effect on specific weight. As specific weight is a key determinant for the price the farmer receives and the overall marketability of the product, farmers need to balance the increase in yield obtained by applying nitrogen with potential detrimental effects on specific weight. On the other hand, the non-significant interaction of hullability and kernel content with increasing levels of nitrogen might indicate higher genotype influence on variety selection when breeding for both quality parameters. There was a non-consistent effect of nitrogen on thousand grain weight, which was positively correlated with grain width explaining the similar response found for both traits to increasing nitrogen. Oil content was negatively affected with increasing nitrogen for all varieties and sites but the opposite effect on β-glucan and protein content was found, which might result in varieties with a more suitable chemical composition for the milling industry and human consumption.

None of the parameters positively affected by increasing levels of nitrogen, i.e. yield, β-glucan and protein content, kernel content and hullability, displayed a plateau and thus it was not possible to calculate the optimal amount of nitrogen to apply for a maximal response. The loss of bimodality in grain and groat area and length at higher nitrogen levels could be a benefit for the milling industry, due to the homogenization of the size of the grain and the groat which could facilitate down-stream processes in the milling industry.

6.3 Grain Development

Despite its economic importance, few studies have investigated so far how development of the grains in the oat panicle affects grain quality parameters of importance for the milling industry and end-users. The characteristics of the panicle influence the variability in grain size and shape between types of grain as found in this thesis (chapter three), and as previously reported (Browne et al., 2006), suggesting that grain quality depends on the development of individual grains and that season, site and variety rather than management conditions, as found for several grain quality parameters under different fertilization levels (chapter four), are crucial factors affecting development and growth and therefore grain quality.

Although there are structural differences between oats and wheat and barley, there is no oat specific grain development guidance against which best management conditions can be applied and adjusted according to benchmarks as quantitative reference points of panicle and grain development. So far, the optimum time being targeted for harvest is more dependent on weather conditions rather than to the best possible values of grain quality parameters which might lead to a better cost-benefit balance for the farmer, the milling industry and end-users. Consequences of harvesting at the incorrect time due to a lack of specific oat grain development guidelines include shorter grain filling periods, not fully mature grain, higher moisture content, and higher variability in grain size and shape, among others.

In chapter five three oat varieties were grown and phenotyped from flowering time to harvest in two field trials under standard management conditions.

Although each variety displayed a unique pattern of development for each trait studied, i.e. kernel content, thousand grain weight, moisture content and grain and groat size and shape, several commonalities were also established.

Primary and secondary grain displayed the same pattern within varieties. There were differences between the top and the bottom of the panicle, i.e. whorls, for all grain quality traits and grain and groat size and shape values. The differences found are explained by the wide range in flowering time observed in oats with the top of the panicle flowering much earlier than those at the base. Therefore, grains at the top of the panicle have longer grain filling periods in comparison with grains at the bottom, resulting in higher values.

Kernel content and thousand grain weight displayed a specific pattern of development for Mascani, whilst Buffalo and Tardis had greater similarity. Grain size and shape were also similar in Buffalo and Tardis reaching maximum values differently to Mascani. It might be interesting to look at the genetic history of the three varieties to investigate possible common genealogies and genes shared that underlie these common pattern developments and the differences found between them. Top of the panicle reached maximum values before lower whorls for all varieties. Rates of change were higher for secondary grain at all stages and for all varieties in comparison with primary grain. Both results suggest that primary grain and top whorls are the first to be fixed with lower whorls and secondary grain more variable in response to environmental conditions.

Frequency distribution histogram analysis showed a reduction in the range of values obtained as development progressed and changes in the bimodal distributions due to differences between the two types of grain at early stages of development for all varieties grain area and shape. However, grain width lost the bimodality character of the distribution by the end of grain development.

These results suggest that grain width is the first grain size trait to be settled in grain development. All varieties reached maximum values in grain width before grain length, suggesting that grain length might have higher influence on final grain area. The independent development found between width and length may reflect differential modulation in growth that can be titled as growth arrest along the main stem of the panicle and along grain axes, at different developmental stages (Gegas *et al.*, 2010).

Groat size and shape for all varieties displayed a stable pattern with reductions in groat area, length and width that resembled reducing moisture content. It might be argued that as the groat develops, the kernel would reach first maximum width values, growing then in length. At later stages a shrinking effect of the seed with the loss of moisture would allow the hull to detach from the groat. This implies that width might act as a a plastic groat size parameter for moisture content inside the husk and therefore being more affected by the loss of moisture content along with secondary grain. This might explain the higher hullability found in secondary grain, previously described with higher grain width than primary grain (Browne et al., 2002).

6.4 Final conclusions

- Environmental influence, management conditions and genetic differences on grain quality parameters, were determined by statistical analysis. Results showed that there was a differential effect of environment on grain chemical and physical parameters. Statistically significantly differences for area, length and width between varieties and locations (p-value <0.05) were found, showing correlation with kernel content, hullability and thousand grain weight

- None of the varieties displayed a superior performance in all quality traits nor did any one site showed a superior performance over all values for all varieties. Interactions found for chemical quality traits between genotype and environment suggest that niche-matching varieties according to the chemical trait of interest could be conducted.

- Environments where the varieties were grown displayed variable grain quality results, suggesting that these sites are more suitable to future further investigations on grain quality differences in terms of genotype by environment interactions.

- Grain quality parameters showed non-linear responses, positive and negative, with increasing levels of nitrogen. Specific weight was lower with higher levels of nitrogen. None of the quality parameters positively affected by increasing levels of nitrogen, i.e. yield, β-glucan and protein content, kernel content and hullability, displayed a plateau and thus it was not possible to calculate the optimal amount of nitrogen to apply for a maximal response.

- Grain development results showed differences between the top and the bottom of the panicle in terms of maturity and also the effect of loss of moisture content during maturation. Each variety showed a unique pattern of development, although some similarities were found. Maximal grain width was reached before maximum grain length with both of them diminishing by final maturity. Groat size parameters showed less variation than grain. This might lead to investigate a more important role of the husk in the variability observed between grain size in all varieties.

- Tardis had consistently lower sensitivity values in general terms and positively affected by increasing levels of nitrogen, allowing choosing as a suitable candidate in breeding programs and to continue investigating the genetic basis of grain quality parameters.

- None of the milling quality parameter showed the best association with the environment. However, hullability was the most consistent through environments and positively affected by increasing levels of nitrogen, representing an interesting milling quality parameter for further investigations, including its association with moisture content and other quality parameters.

- Although a new method to assess milling quality parameters was not found, it is possible to develop a predictive response model. This would include as much phenotypic information from combined the field and laboratory to predict final yield and milling quality parameters.

6.5 Future challenges

Future challenges include determining how much of the observed quality traits' variability was caused by genetic variation and how much by differences in management practices. Those studies would help to determine the best cost-effective management conditions in the region to get the most of each variety. Future experimental trials should be designed to include major areas of crop production and different genotypes along longer periods of time, i.e. more harvest seasons. This would allow a better understanding of genetic and environment interactions and their effects on grain quality parameters and therefore a niche-matching list of varieties across the country could be developed. A farmer could refer to that list when searching for an oat variety, ensuring higher results when farming in a certain area of the country. A milling quality and yield predictive mathematical model could be also developed based on associations between grain and groat size and shape and quality parameters found in this research along with future studies.

Further investigation will be necessary to elaborate a better mathematical model to fit causal relationships between different levels of nitrogen applied and grain quality parameters. An optimum nitrogen rate or plateau above which the cost-benefit enhancing grain quality parameters, remains unclear. Further investigations on the effects of intermediate levels of nitrogen fertilizer area needed to elaborate a comprehensive and mathematically accurate model to establish the relationships between grain size and shape and grain quality parameters under nitrogen fertilizer effects.

At the same time, grain development studies combining grain quality parameters with grain and groat size and shape in oats have been proven to yield interesting associations between the physical basis of grain and groat and milling quality parameters. Further analysis of the data produced is necessary to develop a grain growth model. This model could be compared to similar crops whose inflorescence resembles oats for example rice. Including weather data and metabolomics analysis along grain development would complete also with detailed information, how light, temperature and water availability might affect key developmental processes, crucial for the establishment of flowering time, growing degree days, grain filling period, variation in grain chemical quality traits and composition, among others.

Oats, with its panicle structure and development and the differences between specific varieties, show specific values in terms of size and shape descriptors. These descriptors can be used as variety-specific parameters. Current classification of seed shape and size relies on human skills with a certain level of subjective and requiring experience. This does not describe diversity within and between populations neither between primary and secondary grain (see *Introduction* chapter). Oat processing, and particularly the milling industry, is especially affected by physical parameters of grains and groats and specifically by the differences between primary and secondary grain. Kernel diversity could be defined in terms of image analysis, through size dimensions and shape descriptors.

Quality methods to assess grain quality parameters in crops and in general in food products, is nowadays enhanced by consumers, by their expectations and awareness by influencing changes in quality standards. The high labour costs, variability and inconsistency associated with human inspection accentuate the need for objective measurements systems that guarantee end-users requirements and standards. Automatic inspection systems, such as computer vision, since their origin in the 1960's have been proved to be useful as tool to measure agricultural and food products (Gunasekaran, 2000; Brosnan & Sun, 2004).

Computer aided image analysis, also known as computer vision, is a simple and low-cost method, that can facilitate certain stages of the selection process, similarly to marker assisted selection (MAS). Image analysis is a complementary method along with molecular techniques, allowing preliminary selection of hybrids as a first and accurate contact modality to choose for those varieties that show the best performance in a certain grain quality trait.

Computer vision offers an increasing potential to automate manual grading practices, thus standardising techniques and eliminating human inspection tasks. The current visual classification procedure is demanding, even for trained inspectors because of the wide variation in visual characteristics caused by contrasting class, varietal and environmental effects (Zayas, Martin, Steele, & Katsevich, A. 1996). To date the majority of the morphometric grain test using several shape and colour descriptors have involved the identification of barley and wheat species and varieties (Armstrong , Weiss, Grieg, Dines, Gooden & Aldred, no date; Shouche, , Rastogi, Bhagwat, & Sainis, 2001), evaluations of grain health and seed purity and mechanical damage (Lee, Yan, Wang, Lee and Park, 2011).

A new method able to assess grain quality parameters, identifying not only varieties but primary from secondary grain, would have a deep economic impact not only for the milling industry but also for the end-user.

So far, image analysis in oats concerning this research has been done based on MARVIN (*Chapter Material and methods*). Although effective in measuring grain and groat size and thousand grain weight from individual seeds, the image that it takes is two dimensional. To fully understand the role of grain size parameters in grain quality it is necessary to examine the three dimensional (3-D) structure of the grain. Computerised tomography (CT) scanning uses X-rays to create detailed images and can be used to analyse internal structures. The CT scanning output is a 3D image that allows the calculation of the volume of the seed, and new physical characteristics that may affect important grain quality parameters, e.g. depth of the crease of the grain and the distance between the groat and grain.

Computer vision includes the capture, processing and analyses of images allowing, by a non-destructive method and can be used to assess visual quality characteristics in food products and plant phenotype determination (Brosnan & Sun, 2004). Images are acquired with a physical sensor (Camera, scanner, CT scanning) and through hardware and software, the images are analysed to perform a predefined visual task. The process includes a conversion from images to numerical form which is called digitisation. The image is divided into a two-dimensional grid of small regions containing picture elements defined as pixels by using a vision processor board called a digitiser or frame grabber. Removing defects such as distortion, improper focus, noise, motion and non-uniform lighting, image analysis allows distinguishing an object (region of interest) from the background, and produces quantitative information (Brosnan & Sun, 2004).

One of the key problems in image analysis is the large amount of data related to shape of the studied object that can obstruct or disable the image segmentation. Therefore, it is also necessary to implement image descriptors. These descriptors are rarely individually correlated with the examined shape attributes, so a multivariate analysis is required to clearly discriminate between objects (Wiwart, Zbieta, Lajszner & Graban, 2012).

In a preliminary experiment to determine the potential of the approach, two subsets of oat samples involving four winter oat varieties were analysed using CT scanning and the dimensions obtained compared to those obtained using MARVIN. The scans were generated using a medium resolution (55kV, 200µA, 34mm tube and a 35.2mm FOV) and these specifications determined the after images processing.

Once the images were available they were analysed with *ImageJ* software. The first step was the reconstruction of the 3D image structures. For the first set of samples, i.e. Buffalo (figure 6.1 and 6.2) the whole ear was reconstructed, following the instructions of a pre-made macro by Hughes, Nathan (Hughes, Askew, Scotson, Williams, Sauze, Corke, Doonan & Nibau, 2017). In the second analysis, the segmented grain was reconstructed with a similar macro, but with an extra step in order to eliminate outliers from the final image.

Preliminary results with this method are promising facilitating the measurement of both the grain and groat without direct manipulation of the sample, reducing manual work and keeping the integrity of the sample.

At the same time new parameters could be measured such as the crease depth of the groat (table 6.1 and figure 6.2), the distance between groat and husk, thickness of the husk, etc. All these new parameters might help to elucidate the complex relationships between grain and groat quality traits and grain and groat size and shape.

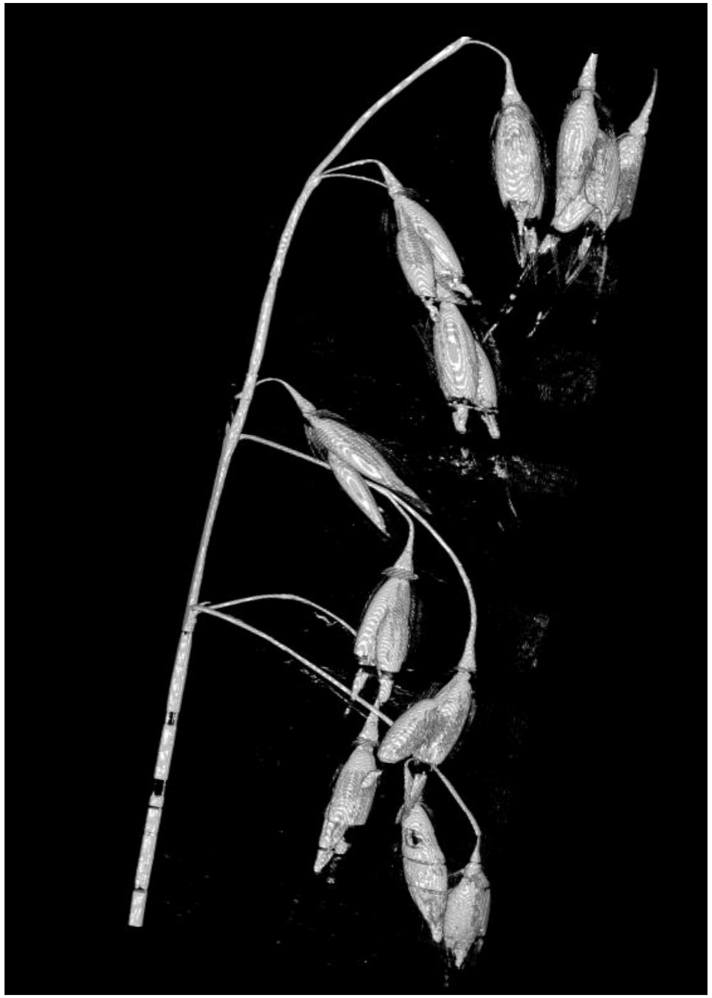
Crease and crease depth (table 6.1 and figure 6.2) might play a key with hullability of the grain, affecting dehulling protocols. This effect could be due to the role of the crease as the dehulling point meaning the space between the husk and the groat that facilitate the dehulling process. Additionally groat β-glucan content could be measure and analyse in association with the thickness of the wall, data that could be extracted from the analysis of groat images, allowing locating the main concentration groat areas, groats with higher content, etc.

Kernel mass, a three-dimensional measurement, may be the best evaluation of kernel size (Doehlert *et al.*, 2004). So far two dimensional images, lacking the third dimension i.e. kernel density, have been unable to provide for this measurement given instead, length, width and from them kernel area. Milling industry processes like sieving, would beneficiate from a better understanding of the kernel depth, being the third dimension essential on its determination.

Table 6.1 *Buffalo's groat size and shape parameters obtained after segmentation and analysis of tri-dimensional and two-dimensional images from CT scanning.*

	Length	Width	Depth	Ratio	Circularity	Volume	Crease Depth	Surface Area	Crease Volume	Axe x	Axe y	Axe z	Spike ID
0	10.52	5.42	3.54	1.94	0.49	866.99	1.02	1.37	0.0006	313.80	408.22	194.78	C0001182
1	9.63	5.11	3.66	1.88	0.70	780.99	0.73	1.35	0.0007	295.10	363.56	353.85	C0001182
2	7.29	3.61	2.93	2.01	0.85	375.98	0.34	0.67	0	291.45	74.72	454.85	C0001182
3	7.01	3.16	3.12	2.21	0.91	338.59	0.14	0.61	0	225.21	402.70	464.50	C0001182
4	7.63	4.00	2.54	1.90	0.80	390.34	0.40	0.74	0.0016	196.61	231.01	714.51	C0001182
5	6.26	6.70	3.64	0.93	0.43	679.72	1.14	1.29	0.0013	262.13	351.33	717.25	C0001182
6	3.04	3.92	2.46	1.01	0.81	198.13	0.29	0.43	0	236.19	242.88	732.43	C0001182

Figure 6.1 *Buffalo's panicle branch as obtained before segmentation analysis to the left, where the husk and rest of the glumes are still present and after segmentation to the right, where the groats are visible. Images obtained by CT scanning*



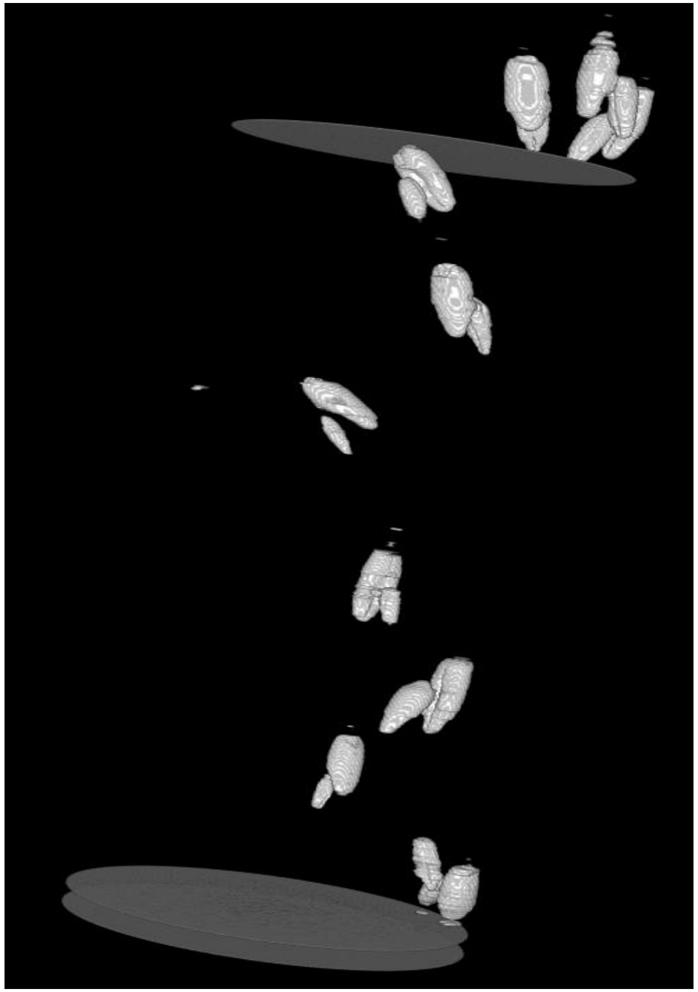
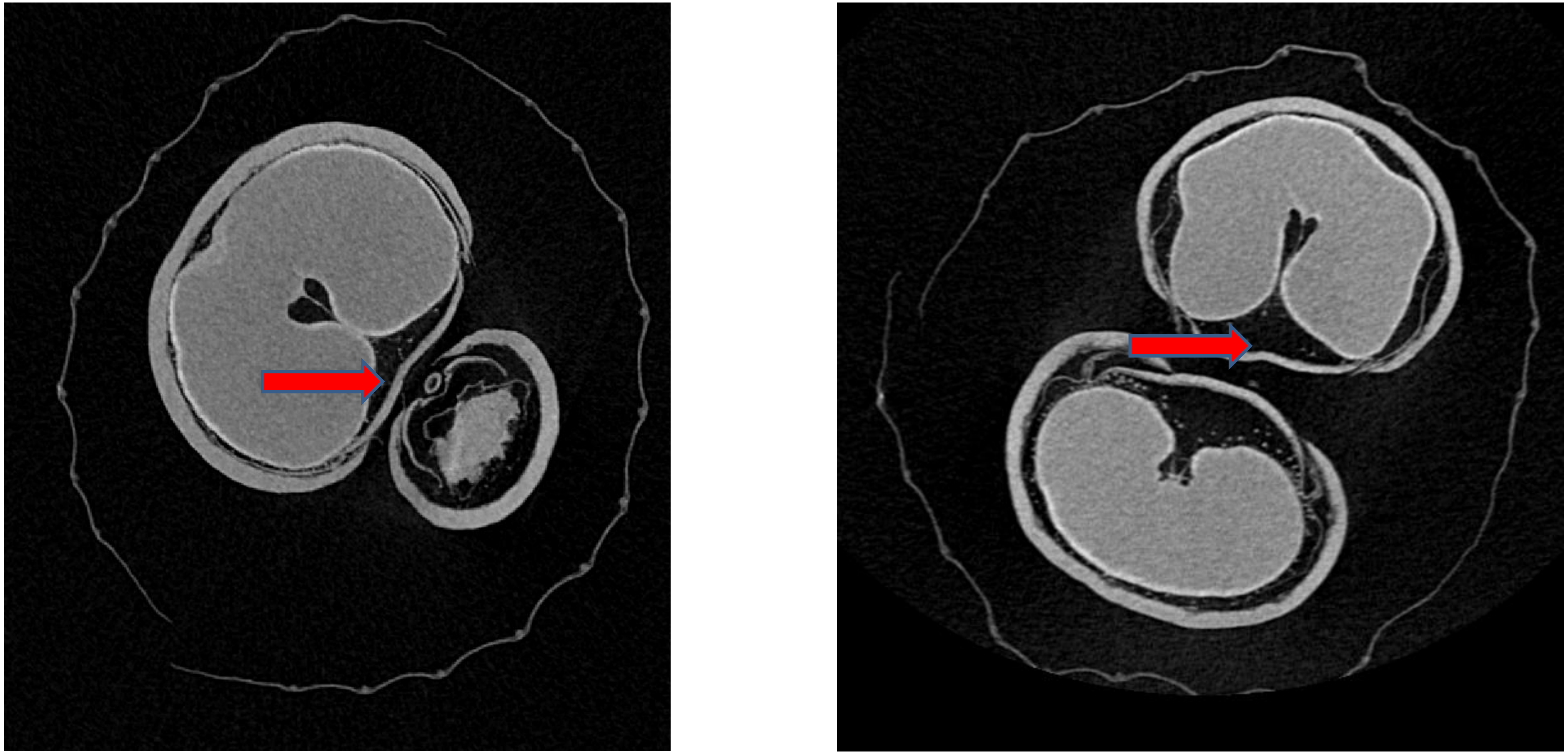


Figure 6.2 *Buffalo's spikelets from the top of the panicle as obtained after segmentation analysis. The glumes, husk and groats can clearly present, red arrows indicate the crease of the groat. Images obtained by CT scanning*



Although still in its first steps, comparisons between data from Buffalo panicles and grain through MARVIN and CT scanning (data not shown) have been inconclusive. They have shown different in grain and groat values due to difficulties in the segmentation process of the CT images.

Thus, there were grain and groat losses, i.e. grain and groat present in the two-dimensional analysis that were not in the three-dimensional output. Groat size measurements were significantly different between the two approaches (p-value<0.001) (data non-shown). Despite this, they suggest interesting new approaches to investigate new shape traits.

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Appendix

Chapter Three. Genetic by environment effects on grain quality parameters

Tables 3.24.b *Pearson´s correlation coefficients (p-value<0.001) between quality traits and grain and groat size of each of the four winter oat varieties. Green numbers show negative correlations whilst red numbers show positive correlations (p*value<0.001).*

	Balado *Oil*	**Gerald** *Oil*	**Mascani** *Oil*	**Tardis** *Oil*	**Balado** *Protein*	**Gerald** *Protein*	**Mascani** *Protein*	**Tardis** *Protein*	**Balado** *B-Glucan*	**Gerald** *B-Glucan*	**Mascani** *B-Glucan*	**Tardis** *B-Glucan*
Oil	1.00	1.00	1.00	1.00								
Protein	-0.53	-0.21	-0.44	-0.31	1.00	1.00	1.00	1.00				
B-Glucan	-0.35	-0.20	-0.75	-0.83	0.33	0.07	0.64	0.42	1.00	1.00	1.00	1.00
Kernel content	-0.73	-0.48	-0.20	-0.58	0.23	0.22	0.17	-0.28	0.15	-0.06	0.18	0.35
Hullability	-0.73	-0.14	-0.26	0.00	0.58	-0.04	-0.43	-0.10	0.02	0.14	-0.02	0.08
Specific weight	-0.37	-0.19	0.39	-0.09	-0.29	-0.08	-0.16	-0.15	0.00	-0.23	-0.31	-0.13
Yield	0.70	0.38	0.59	0.39	-0.55	-0.33	-0.49	-0.26	-0.26	-0.35	-0.58	-0.41

TGW	-0.62	-0.51	0.22	0.10	-0.10	-0.19	-0.50	-0.45	-0.04	0.02	-0.43	-0.28
Grain n^o/m^2	0.81	0.58	0.56	0.32	-0.34	-0.17	-0.33	0.02	-0.20	-0.30	-0.46	-0.25
Area	-0.66	-0.79	-0.78	-0.59	0.19	0.25	0.28	-0.05	0.47	0.18	0.54	0.32
Width	-0.51	-0.39	0.37	0.23	-0.22	-0.30	-0.68	-0.52	-0.10	-0.05	-0.58	-0.40
Length	-0.37	-0.66	-0.80	-0.71	0.34	0.39	0.45	0.20	0.67	0.23	0.67	0.53
Area Groat	-0.88	0.42	0.77	0.64	0.30	-0.47	-0.57	-0.35	0.18	-0.23	-0.73	-0.58
width Groat	-0.59	-0.63	-0.21	-0.27	-0.17	0.08	0.14	-0.07	-0.10	-0.03	0.03	0.06
Length Groat	-0.92	-0.37	0.58	0.11	0.61	-0.36	-0.54	-0.38	0.37	-0.02	-0.68	-0.27
Groat Ratio	0.24	-0.68	-0.68	-0.59	-0.83	0.36	0.65	0.31	-0.48	-0.03	0.60	0.39
Grain Density	-0.13	0.37	0.77	0.57	-0.26	-0.69	-0.74	-0.67	-0.58	0.00	-0.79	-0.60
Circularity	0.13	0.23	0.76	0.49	-0.37	-0.43	-0.50	-0.35	-0.67	-0.13	-0.69	-0.45
Compactness	-0.14	0.50	0.76	0.66	0.37	-0.43	-0.50	-0.27	0.66	-0.23	-0.69	-0.55
Grain Ratio	0.03	-0.51	-0.77	-0.67	-0.40	0.44	0.50	0.28	-0.62	0.23	0.69	0.55

*__Tables 3.24.c__ Pearson´s correlation coefficients (p-value<0.001) between quality traits and grain and groat size of each of the four winter oat varieties. Green numbers show negative correlations whilst red numbers show positive correlations (p*value<0.001).*

	Balado *Kernel content*	Gerald *Kernel content*	Mascani *Kernel content*	Tardis *Kernel content*	Balado *Hullability*	Gerald *Hullability*	Mascani *Hullability*	Tardis *Hullability*
Kernel content	1.00	1.00	1.00	1.00				
Hullability	0.47	0.54	0.37	0.29	1.00	1.00	1.00	1.00
Specific weight	0.68	0.49	-0.32	0.24	0.09	0.00	-0.03	0.03
Yield	-0.45	-0.52	-0.75	-0.35	-0.75	-0.57	-0.34	-0.26
TGW	0.82	0.66	0.12	0.28	0.39	0.18	0.43	0.20
Grain n^{o}/m^{2}	-0.73	-0.74	-0.78	-0.46	-0.70	-0.54	-0.47	-0.31
Area	0.74	0.33	-0.03	0.42	0.16	-0.21	0.11	-0.36

Width	0.71	0.58	0.12	0.18	0.33	0.15	0.50	0.14
Length	0.38	0.01	-0.11	0.32	-0.14	-0.39	-0.11	-0.50
Area Groat	0.86	0.27	0.14	-0.18	0.59	0.42	0.24	0.45
width Groat	0.74	0.65	0.06	0.37	0.36	0.23	0.12	0.16
Length Groat	0.80	0.47	-0.05	0.17	0.65	0.11	0.19	0.04
Groat Ratio	0.04	0.60	0.16	0.45	-0.24	0.20	-0.03	0.16
Grain Density	0.31	-0.20	-0.12	-0.15	0.37	-0.10	0.14	-0.07
Circularity	-0.06	0.41	0.11	-0.01	0.29	0.45	0.16	0.50
Compactness	0.10	0.21	0.16	-0.24	-0.27	0.46	0.21	0.49
Grain Ratio	0.09	-0.17	-0.14	0.26	0.30	-0.46	-0.18	-0.49

Tables 3.24.d *Pearson´s correlation coefficients (p-value<0.001) between quality traits and grain and groat size of each of the four winter oat varieties. Green numbers show negative correlations whilst red numbers show positive correlations (p*value<0.001).*

	Balado	**Gerald**	**Mascani**	**Tardis**	**Balado**	**Gerald**	**Mascani**	**Tardis**
	Sp Wt	*Sp Wt*	*Sp Wt*	*Sp Wt*	*Yield*	*Yield*	*Yield*	*Yield*
Specific weight	1.00	1.00	1.00	1.00				
Yield	-0.13	0.25	0.42	0.36	1.00	1.00	1.00	1.00
TGW	0.85	0.67	0.25	0.45	-0.34	-0.09	0.13	-0.02
Grain n^o/m^2	-0.50	-0.09	0.35	0.10	0.89	0.89	0.96	0.89
Area	0.68	0.22	-0.19	0.06	-0.42	-0.19	-0.26	-0.12
Width	0.82	0.73	0.30	0.41	-0.25	0.04	0.22	0.07
Length	0.23	-0.12	-0.25	-0.10	-0.24	-0.19	-0.25	-0.10
Area Groat	0.67	0.44	0.29	0.24	-0.56	0.17	0.26	0.11
width Groat	0.79	0.57	0.05	0.46	-0.26	-0.12	-0.08	-0.23

Length Groat	0.47	0.74	0.40	0.47	-0.68	0.18	0.38	0.08
Groat Ratio	0.45	0.35	-0.24	0.43	0.39	-0.28	-0.47	-0.47
Grain Density	0.33	0.28	0.40	0.19	0.00	0.43	0.52	0.45
Circularity	0.10	0.43	0.29	0.38	0.08	0.01	0.27	0.03
Compactness	-0.07	0.34	0.26	0.15	-0.09	0.15	0.22	0.09
Grain Ratio	0.26	-0.30	-0.25	-0.12	0.06	-0.16	-0.23	-0.07

Tables 3.24.e *Pearson´s correlation coefficients (p-value<0.001) between quality traits and grain and groat size of each of the four winter oat varieties. Green numbers show negative correlations whilst red numbers show positive correlations (p*value<0.001).*

	Balado	**Gerald**	**Mascani**	**Tardis**	**Balado**	**Gerald**	**Mascani**	**Tardis**
	TGW	*TGW*	*TGW*	*TGW*	*grain n^o/m^2*	*grain n^o/m^2*	*grain n^o/m^2*	*grain n^o/m^2*
TGW	1.00	1.00	1.00	1.00				
Grain n^o/m^2	-0.72	-0.53	-0.15	-0.47	1.00	1.00	1.00	1.00
Area	0.70	0.56	0.11	0.33	-0.67	-0.45	-0.31	-0.28
Width	0.96	0.96	0.93	0.97	-0.63	-0.40	-0.03	-0.38
Length	0.14	0.12	-0.27	-0.32	-0.29	-0.25	-0.19	0.04
Area Groat	0.89	0.33	0.50	0.64	-0.84	0.03	0.13	-0.18
width Groat	0.97	0.85	0.56	0.81	-0.64	-0.49	-0.23	-0.57
Length Groat	0.67	0.91	0.86	0.91	-0.82	-0.26	0.15	-0.34
Groat Ratio	0.44	0.61	-0.09	0.43	0.08	-0.52	-0.44	-0.60
Grain Density	0.52	0.17	0.60	0.68	-0.22	0.29	0.36	0.08
Circularity	0.25	0.48	0.60	0.66	-0.01	-0.18	0.12	-0.27

Compactness	-0.21	0.20	0.44	0.54	-0.01	0.07	0.11	-0.15
Grain Ratio	0.42	-0.15	-0.41	-0.51	-0.11	-0.10	-0.13	0.15

Tables 3.24.d *Pearson´s correlation coefficients (p-value<0.001) between quality traits and grain and groat size of each of the four winter oat varieties. Green numbers show negative correlations whilst red numbers show positive correlations (p*value<0.001).*

	Balado *Area*	**Gerald** *Area*	**Mascani** *Area*	**Tardis** *Area*	**Balado** *Width*	**Gerald** *Width*	**Mascani** *Width*	**Tardis** *Width*	**Balado** *Length*	**Gerald** *Length*	**Mascani** *Length*	**Tardis** *Length*
Area	1.00	1.00	1.00	1.00								
Width	0.61	0.46	-0.08	0.27	1.00	1.00	1.00	1.00				
Length	0.79	0.87	0.91	0.75	0.02	-0.01	-0.44	-0.38	1.00	1.00	1.00	1.00
Area Groat	0.78	-0.56	-0.77	-0.47	0.80	0.47	0.67	0.69	0.33	-0.89	-0.96	-0.93
width Groat	0.61	0.65	0.50	0.38	0.97	0.80	0.35	0.75	0.03	0.28	0.30	-0.12
Length Groat	0.77	0.37	-0.26	0.26	0.53	0.93	0.86	0.92	0.51	-0.07	-0.56	-0.32
Groat Ratio	-0.07	0.71	0.71	0.36	0.59	0.52	-0.32	0.33	-0.48	0.48	0.73	0.15
Grain Density	-0.20	-0.39	-0.59	0.02	0.49	0.29	0.74	0.78	-0.67	-0.55	-0.80	-0.47
Circularity	-0.49	-0.44	-0.71	-0.47	0.36	0.50	0.66	0.64	-0.92	-0.76	-0.90	-0.86
Compactness	0.53	-0.66	-0.80	-0.55	-0.30	0.33	0.60	0.59	0.94	-0.94	-0.98	-0.96
Grain Ratio	-0.32	0.70	0.83	0.59	0.55	-0.29	-0.57	-0.56	-0.82	0.96	0.98	0.97

Tables 3.24.e *Pearson´s correlation coefficients (p-value<0.001) between quality traits and grain and groat size of each of the four winter oat varieties. Green numbers show negative correlations whilst red numbers show positive correlations (p*value<0.001).*

	Balado *Area Groat*	Gerald *Area Groat*	Mascani *Area Groat*	Tardis *Area Groat*	Balado *width Groat*	Gerald *width Groat*	Mascani *width Groat*	Tardis *width Groat*	Balado *Length Groat*	Gerald *Length Groat*	Mascani *Length Groat*	Tardis *Length Groat*
Area Groat												
width Groat	1.00	1.00	1.00	1.00								
Length Groat	0.86	0.77	0.47	0.88	1.00	1.00	1.00	1.00				
Groat Ratio	0.92	0.90	0.70	0.83	0.59	0.44	-0.28	0.49	1.00	1.00	1.00	1.00
Grain Density	0.06	-0.24	-0.15	0.34	0.56	0.43	0.79	0.74	-0.33	-0.62	-0.80	-0.23
Circularity	0.32	0.22	0.03	0.46	0.54	0.55	0.81	0.60	0.09	-0.07	-0.57	0.16
Compactness	0.02	0.01	-0.18	0.30	0.34	0.35	0.67	0.50	-0.24	-0.26	-0.70	-0.05
Grain Ratio	0.01	0.03	0.21	-0.28	-0.30	-0.32	-0.65	-0.48	0.26	0.30	0.70	0.07

Tables 3.24.e *Pearson´s correlation coefficients (p-value<0.001) between quality traits and grain and groat size of each of the four winter oat varieties. Green numbers show negative correlations whilst red numbers show positive correlations (p*value<0.001).*

	Balado	Gerald	Mascani	Tardis	Balado	Gerald	Mascani	Tardis
	Groat Ratio	*Groat Ratio*	*Groat Ratio*	*Groat Ratio*	*Grain Density*	*Grain Density*	*Grain Density*	*Grain Density*
Groat Ratio	1.00	1.00	1.00	1.00	1.00			
Grain Density	0.53	0.54	0.86	0.86	0.82	1.00	1.00	1.00
Circularity	0.64	0.57	0.85	0.85	-0.82	0.86	0.93	0.93
Compactness	-0.62	-0.58	-0.84	-0.84	0.83	-0.86	-0.93	-0.93

Table 4.12.a *Proportions, mean and standard deviation values from bimodality distribution analysis of each variety at each level of fertilization at IBERS 2014*

IBERS 2014			*Grain*						*Groats*					
Grain	*Trait*	*N level*	*Proportion 2*	*Proportion 1*	*Mean 2º*	*sd 2*	*Mean 1º*	*sd 1*	*Proportion 2º*	*Proportion 1º*	*Mean 2º*	*sd 2º*	*Mean 1º*	*sd 1º*
Balado	Area mm^2	0	0.48	0.52	21.26	2.94	32.65	2.96	0.60	0.40	13.68	1.33	19.06	1.63
		1	0.51	0.49	24.20	3.19	34.58	2.61	0.63	0.37	15.32	1.21	20.21	1.65
		2	0.51	0.49	24.96	3.54	34.81	2.89	0.71	0.29	15.86	1.29	20.90	2.41
		3	0.46	0.54	24.28	3.46	34.20	3.43	0.49	0.51	15.04	2.15	19.75	2.05
		4	0.54	0.46	25.60	4.02	35.59	3.28	0.76	0.24	17.19	1.35	22.09	2.46
		5												
Balado	Length mm	0	0.47	0.53	9.71	1.10	13.42	0.97	0.58	0.42	6.34	0.40	7.73	0.53
		1	0.54	0.46	10.98	1.16	14.20	0.68	0.65	0.35	6.93	0.39	8.19	0.56
		2	0.55	0.45	11.39	1.28	14.48	0.84	0.88	0.12	7.42	0.25	8.52	0.83
		3	0.53	0.47	11.42	1.25	14.58	0.88	0.52	0.48	7.08	0.60	8.19	0.68
		4	0.62	0.38	11.86	1.47	14.82	0.84	0.76	0.24	7.61	0.40	8.69	0.71
		5												
Balado	Width mm	0	0.45	0.55	2.96	0.22	3.42	0.17	0.63	0.37	2.66	0.13	2.96	0.17
		1	0.20	0.80	2.88	0.17	3.39	0.19	0.86	0.14	2.77	0.06	3.03	0.15
		2	0.18	0.82	2.88	0.13	3.34	0.19	0.07	0.93	2.35	0.19	2.78	0.10

		3	0.21	0.79	2.80	0.18	3.31	0.21	0.05	0.95	2.28	0.20	2.75	0.06
		4	0.21	0.79	2.84	0.19	3.33	0.21	0.10	0.90	2.45	0.19	2.80	0.14
		5												
Gerald	Area mm^2	0	0.46	0.54	18.37	1.89	28.34	1.99	0.53	0.47	11.46	1.03	16.34	1.14
		1	0.48	0.52	20.05	2.31	29.83	2.09	0.54	0.46	12.62	0.94	17.09	1.36
		2	0.48	0.52	19.99	2.61	29.72	2.21	0.53	0.47	12.34	1.15	16.93	1.59
		3	0.47	0.53	19.96	2.56	29.69	2.25	0.52	0.48	12.51	1.30	17.37	1.59
		4	0.47	0.53	20.65	2.61	30.27	2.57	0.60	0.40	13.60	1.17	17.91	1.93
		5												
Gerald	Length mm	0	0.46	0.54	8.70	0.71	11.76	0.72	0.53	0.47	5.64	0.34	6.96	0.40
		1	0.50	0.50	9.34	0.88	12.27	0.64	0.51	0.49	5.98	0.35	7.15	0.46
		2	0.49	0.51	9.30	0.94	12.19	0.70	0.53	0.47	6.00	0.38	7.19	0.55
		3	0.49	0.51	9.44	0.98	12.38	0.66	0.43	0.57	5.95	0.49	7.28	0.47
		4	0.47	0.53	9.65	0.94	12.49	0.77	0.47	0.53	6.17	0.41	7.31	0.53
		5												
Gerald	Width mm	0	0.71	0.29	3.04	0.23	3.33	0.08	0.65	0.35	2.56	0.09	2.86	0.15
		1	0.77	0.23	3.11	0.24	3.38	0.08	0.40	0.60	2.54	0.10	2.82	0.09
		2	0.72	0.28	3.07	0.24	3.37	0.09	0.73	0.27	2.60	0.08	2.85	0.18
		3	0.73	0.27	3.04	0.24	3.35	0.10	0.62	0.38	2.56	0.11	2.84	0.17
		4	0.49	0.51	2.99	0.19	3.33	0.15	0.47	0.53	2.56	0.12	2.84	0.14
		5												

Mascani	Area mm^2	0	0.48	0.52	20.94	2.71	32.22	2.28	0.50	0.50	13.75	1.03	18.85	1.20
		1	0.50	0.50	22.73	2.86	33.70	2.58	0.52	0.48	14.62	1.15	19.57	1.46
		2	0.46	0.54	22.61	2.71	33.22	2.55	0.52	0.48	14.89	1.31	19.94	1.68
		3	0.51	0.49	23.29	3.43	34.36	2.72	0.56	0.44	15.57	1.35	20.45	1.85
		4	0.51	0.49	24.48	3.17	35.07	2.88	0.66	0.34	16.63	1.17	21.63	2.37
		5												
Mascani	Length mm	0	0.55	0.45	10.01	1.22	13.16	0.62	0.51	0.49	6.30	0.31	7.66	0.43
		1	0.58	0.42	10.65	1.14	13.57	0.69	0.53	0.47	6.62	0.34	7.88	0.52
		2	0.52	0.48	10.53	0.99	13.44	0.72	0.51	0.49	6.65	0.39	8.00	0.52
		3	0.70	0.30	11.26	1.69	14.00	0.67	0.64	0.36	7.05	0.36	8.26	0.64
		4	0.61	0.39	11.42	1.22	14.24	0.77	0.71	0.29	7.30	0.30	8.42	0.74
		5												
Mascani	Width mm	0	0.35	0.65	2.93	0.19	3.40	0.15	0.39	0.61	2.65	0.10	2.90	0.09
		1	0.26	0.74	2.93	0.17	3.40	0.17	0.57	0.43	2.70	0.08	2.95	0.11
		2	0.38	0.62	3.02	0.19	3.42	0.14	0.58	0.42	2.72	0.08	2.95	0.12
		3	0.65	0.35	3.16	0.28	3.45	0.11	0.64	0.36	2.73	0.09	2.95	0.13
		4	0.38	0.62	3.05	0.21	3.42	0.15	0.65	0.35	2.76	0.11	2.96	0.17
		5												
Tardis	Area mm^2	0	0.45	0.55	23.10	2.49	33.00	2.62	0.57	0.43	14.57	1.19	19.26	1.25
		1	0.53	0.47	25.16	3.43	34.72	2.69	0.71	0.29	15.99	1.11	20.41	1.73
		2	0.72	0.28	25.59	5.53	35.94	2.31	0.88	0.12	16.40	0.95	21.29	2.30
		3	0.64	0.36	25.73	5.13	36.19	2.69	0.82	0.18	16.54	1.05	21.10	2.49

		4	0.71	0.29	26.66	6.10	36.81	2.51	0.82	0.18	17.08	1.04	21.25	2.46
		5												
Tardis	Length mm	0	0.48	0.52	10.70	0.98	13.87	0.94	0.65	0.35	6.90	0.41	7.92	0.52
		1	0.62	0.38	11.69	1.33	14.59	0.77	0.95	0.05	7.41	0.36	8.34	0.63
		2	0.77	0.23	11.93	2.05	14.90	0.70	0.03	0.97	5.69	0.71	7.60	0.35
		3	0.73	0.27	12.24	2.00	15.30	0.74	0.14	0.86	6.43	0.65	7.74	0.62
		4	0.77	0.23	12.39	2.22	15.60	0.88	0.06	0.94	6.07	0.64	7.81	0.32
		5												
Tardis	Width mm	0	0.81	0.19	3.20	0.22	3.42	0.06	0.46	0.54	2.66	0.10	2.88	0.10
		1	0.48	0.52	3.15	0.20	3.37	0.14	0.90	0.10	2.78	0.05	3.02	0.13
		2	0.12	0.88	2.67	0.12	3.25	0.21	0.08	0.92	2.44	0.13	2.76	0.05
		3	0.08	0.92	2.68	0.11	3.23	0.21	0.84	0.16	2.70	0.06	2.92	0.14
		4	0.13	0.87	2.68	0.13	3.25	0.21	0.08	0.92	2.45	0.13	2.77	0.05
		5												

Table 4.12.b *Proportions, mean and standard deviation values from bimodality distribution analysis of each variety at each level of fertilization at ADAS 2014*

ADAS 2014			*Grain*						*Groats*					
Varieties	*Traits*	*N level*	*Proportion 2º*	*Proportion 1º*	*Mean 2º*	*sd 2º*	*Mean 1º*	*sd 1º*	*Proportion 2º*	*Proportion 1º*	*Mean 2º*	*sd 2º*	*Mean 1º*	*sd 1º*
Balado	Area mm2	0	0.46	0.54	21.86	2.38	33.47	3.14	0.70	0.30	13.65	1.67	19.15	1.37
		1	0.48	0.52	22.58	2.74	33.82	2.65	0.66	0.34	14.20	1.96	19.33	1.34
		2	0.46	0.54	22.47	2.83	33.55	2.87	0.60	0.40	14.00	1.91	19.12	1.67
		3	0.51	0.49	23.36	3.25	34.42	2.75	0.69	0.31	14.71	2.23	20.06	1.39
		4	0.48	0.52	23.75	3.05	34.64	2.87	0.66	0.34	15.22	2.17	20.23	1.71
		5	0.45	0.55	22.80	3.00	34.05	3.50	0.72	0.28	14.81	2.38	20.35	1.61
Balado	Length mm	0	0.46	0.54	10.50	0.82	14.16	1.01	0.76	0.24	6.66	0.59	8.01	0.34
		1	0.51	0.49	10.70	1.04	14.26	0.76	0.70	0.30	6.72	0.65	8.00	0.44
		2	0.48	0.52	10.54	1.06	14.08	0.79	0.66	0.34	6.76	0.68	8.03	0.53
		3	0.53	0.47	10.90	1.19	14.34	0.80	0.78	0.22	7.01	0.79	8.31	0.41
		4	0.51	0.49	11.28	1.16	14.66	0.85	0.92	0.08	7.40	0.83	8.48	0.31
		5	0.52	0.48	11.20	1.26	14.82	0.82	0.75	0.25	7.12	0.73	8.56	0.49
Balado	Width mm	0	0.39	0.61	2.91	0.22	3.31	0.21	0.67	0.33	2.53	0.17	2.89	0.15
		1	0.50	0.50	3.02	0.21	3.39	0.15	0.82	0.18	2.65	0.21	2.92	0.11
		2	0.37	0.63	2.95	0.18	3.35	0.18	0.63	0.37	2.57	0.20	2.86	0.15
		3	0.38	0.62	2.95	0.20	3.35	0.17	0.44	0.56	2.52	0.18	2.82	0.17

		4	0.66	0.34	3.08	0.24	3.38	0.14	0.77	0.23	2.62	0.20	2.91	0.14
		5	0.33	0.67	2.86	0.17	3.24	0.20	0.79	0.21	2.51	0.22	2.84	0.13
Gerald	Area mm2	0	0.42	0.58	18.63	1.88	29.40	2.39	0.56	0.44	11.39	1.21	16.15	1.20
		1	0.42	0.58	18.87	2.14	29.17	2.04	0.48	0.52	11.24	1.24	15.77	1.11
		2	0.43	0.57	17.95	2.12	28.46	2.44	0.52	0.48	10.89	1.23	15.72	1.24
		3	0.43	0.57	18.14	2.26	28.44	2.66	0.46	0.54	11.10	1.29	15.84	1.58
		4	0.40	0.60	19.06	2.26	28.91	2.55	0.47	0.53	11.55	1.49	16.05	1.40
		5	0.39	0.61	18.35	1.94	27.98	2.76	0.49	0.51	11.04	1.41	15.58	1.52
Gerald	Length mm	0	0.42	0.58	9.36	0.77	12.66	0.75	0.54	0.46	5.77	0.42	6.98	0.43
		1	0.43	0.57	9.45	0.90	12.58	0.61	0.40	0.60	5.65	0.39	6.87	0.45
		2	0.44	0.56	8.99	0.90	12.30	0.74	0.51	0.49	5.59	0.44	6.98	0.45
		3	0.44	0.56	9.07	0.89	12.34	0.82	0.39	0.61	5.59	0.44	6.89	0.56
		4	0.42	0.58	9.42	0.92	12.53	0.75	0.35	0.65	5.67	0.44	6.95	0.52
		5	0.41	0.59	9.33	0.82	12.54	0.77	0.47	0.53	5.73	0.49	7.06	0.51
Gerald	Width mm	0	0.43	0.57	2.82	0.17	3.24	0.15	0.64	0.36	2.45	0.16	2.79	0.11
		1	0.43	0.57	2.83	0.16	3.23	0.12	0.67	0.33	2.48	0.18	2.77	0.08
		2	0.52	0.48	2.84	0.20	3.22	0.13	0.61	0.39	2.40	0.15	2.75	0.09
		3	0.72	0.28	2.93	0.24	3.24	0.10	0.58	0.42	2.43	0.16	2.76	0.11
		4	0.43	0.57	2.84	0.17	3.18	0.15	0.65	0.35	2.46	0.17	2.76	0.10
		5	0.47	0.53	2.78	0.16	3.10	0.16	0.80	0.20	2.42	0.18	2.71	0.11
Mascani	Area mm2	0	0.49	0.51	21.76	2.33	32.98	2.60	0.58	0.42	13.81	1.54	18.66	1.17
		1	0.54	0.46	23.41	2.76	34.13	2.52	0.60	0.40	14.71	1.89	19.50	1.34

		2	0.48	0.52	22.46	2.82	33.35	2.43	0.56	0.44	14.33	1.93	19.13	1.23
		3	0.51	0.49	22.56	2.67	33.38	2.50	0.60	0.40	14.80	1.90	19.90	1.22
		4	0.49	0.51	23.42	2.75	33.93	2.49	0.62	0.38	15.12	2.04	19.86	1.27
		5	0.53	0.47	22.88	3.05	33.20	2.75	0.73	0.27	15.24	2.38	20.05	1.17
Mascani	Length mm	0	0.52	0.48	10.58	0.92	13.86	0.79	0.64	0.36	6.60	0.55	7.71	0.35
		1	0.58	0.42	10.94	1.10	14.00	0.79	0.73	0.27	6.94	0.72	7.99	0.39
		2	0.51	0.49	10.55	1.07	13.64	0.77	0.74	0.26	6.93	0.79	7.96	0.32
		3	0.55	0.45	10.65	1.03	13.83	0.73	0.71	0.29	6.93	0.71	8.15	0.36
		4	0.54	0.46	10.97	1.08	14.05	0.69	0.75	0.25	7.09	0.73	8.19	0.33
		5	0.57	0.43	10.85	1.14	13.98	0.73	0.76	0.24	7.04	0.75	8.20	0.35
Mascani	Width mm	0	0.47	0.53	2.96	0.19	3.36	0.14	0.68	0.32	2.63	0.17	2.88	0.10
		1	0.47	0.53	3.06	0.18	3.41	0.14	0.70	0.30	2.67	0.17	2.90	0.10
		2	0.68	0.32	3.13	0.25	3.43	0.10	0.70	0.30	2.64	0.17	2.87	0.09
		3	0.72	0.28	3.14	0.25	3.41	0.10	0.75	0.25	2.69	0.18	2.90	0.09
		4	0.23	0.77	2.93	0.14	3.33	0.16	0.65	0.35	2.63	0.17	2.86	0.10
		5	0.22	0.78	2.86	0.16	3.25	0.19	0.66	0.34	2.59	0.18	2.81	0.12
Tardis	Area mm2	0	0.47	0.53	23.16	2.83	33.55	2.54	0.35	0.65	14.29	0.97	15.73	2.60
		1	0.51	0.49	23.97	3.35	34.01	2.46	0.83	0.17	15.08	2.44	19.63	1.13
		2	0.52	0.48	24.00	3.20	33.92	2.57	0.80	0.20	15.29	2.37	19.70	1.07
		3	0.53	0.47	24.51	3.41	34.20	2.51	0.90	0.10	15.66	2.57	20.70	1.03
		4	0.59	0.41	25.38	4.28	35.40	2.52	0.88	0.12	15.81	2.90	20.64	0.99
		5	0.62	0.38	25.00	4.45	35.23	2.66	0.87	0.13	15.76	2.75	20.64	1.07

Tardis	Length mm	0	0.50	0.50	11.62	1.21	14.87	0.76	0.14	0.86	6.98	0.26	7.07	0.68
		1	0.60	0.40	11.86	1.52	14.86	0.71	0.05	0.95	5.45	0.21	7.34	0.73
		2	0.59	0.41	11.73	1.44	14.78	0.70	0.04	0.96	5.60	0.25	7.41	0.72
		3	0.63	0.37	12.01	1.51	14.88	0.66	0.08	0.92	5.77	0.28	7.62	0.74
		4	0.65	0.35	12.23	1.76	15.18	0.71	0.07	0.93	5.60	0.30	7.58	0.77
		5	0.73	0.27	12.29	1.82	15.30	0.68	0.05	0.95	5.59	0.25	7.56	0.78
Tardis	Width mm	0	0.83	0.17	3.08	0.25	3.28	0.09	0.03	0.97	2.26	0.05	2.64	0.15
		1	0.27	0.73	2.90	0.16	3.26	0.17	0.07	0.93	2.23	0.09	2.67	0.17
		2	0.71	0.29	3.07	0.20	3.36	0.11	0.08	0.92	2.30	0.11	2.69	0.16
		3	0.75	0.25	3.10	0.21	3.38	0.10	0.07	0.93	2.25	0.10	2.66	0.16
		4	0.63	0.37	3.07	0.21	3.34	0.13	0.10	0.90	2.23	0.09	2.68	0.17
		5	0.87	0.13	3.08	0.25	3.38	0.07	0.51	0.49	2.48	0.18	2.74	0.14

Figure 4.2*. *Frequency of individual grain and groat length and width of Balado, at ADAS 2015 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

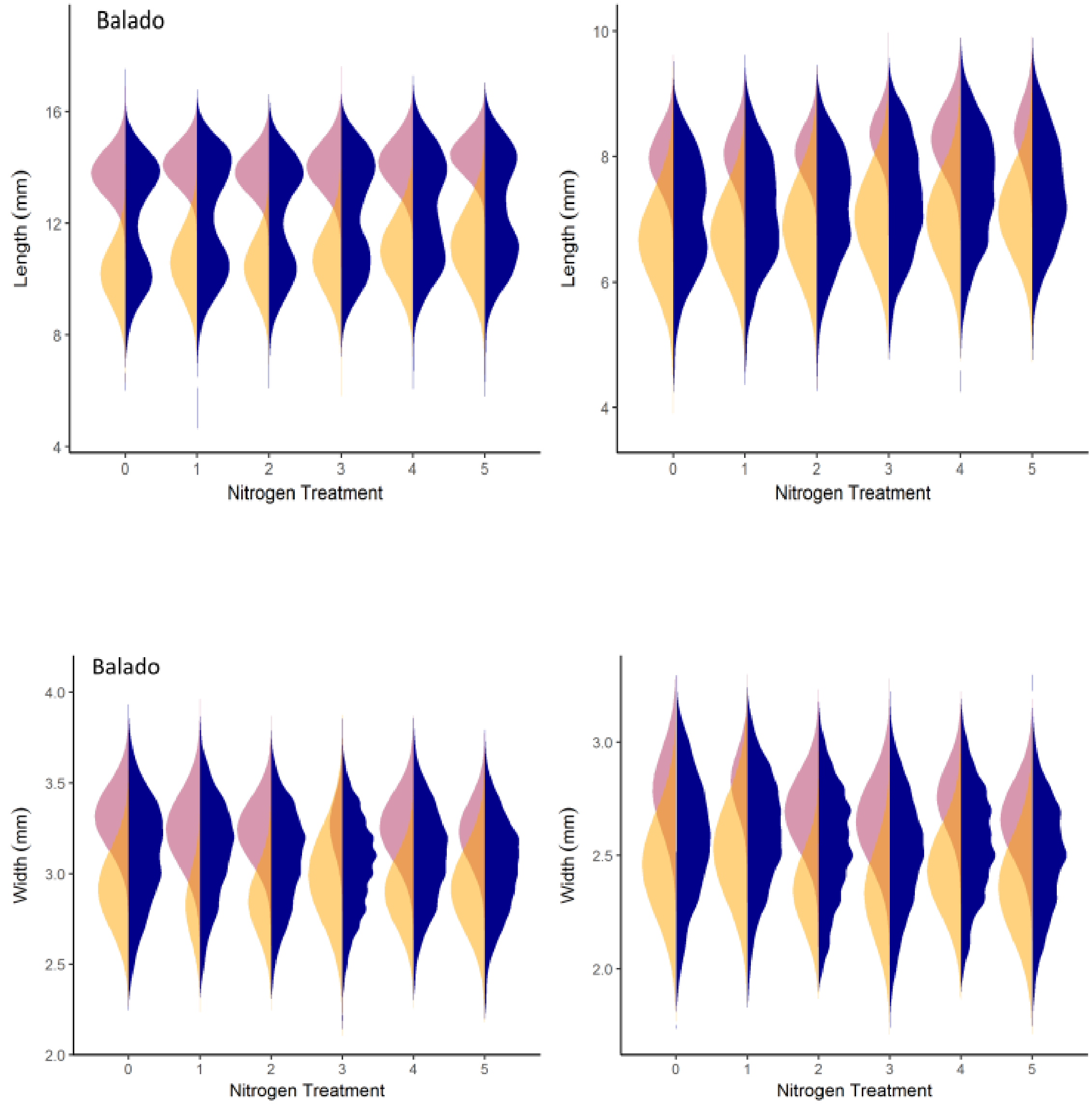


Figure 4.2*. *Frequency of individual grain and groat length and width of Gerald, at ADAS 2015 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

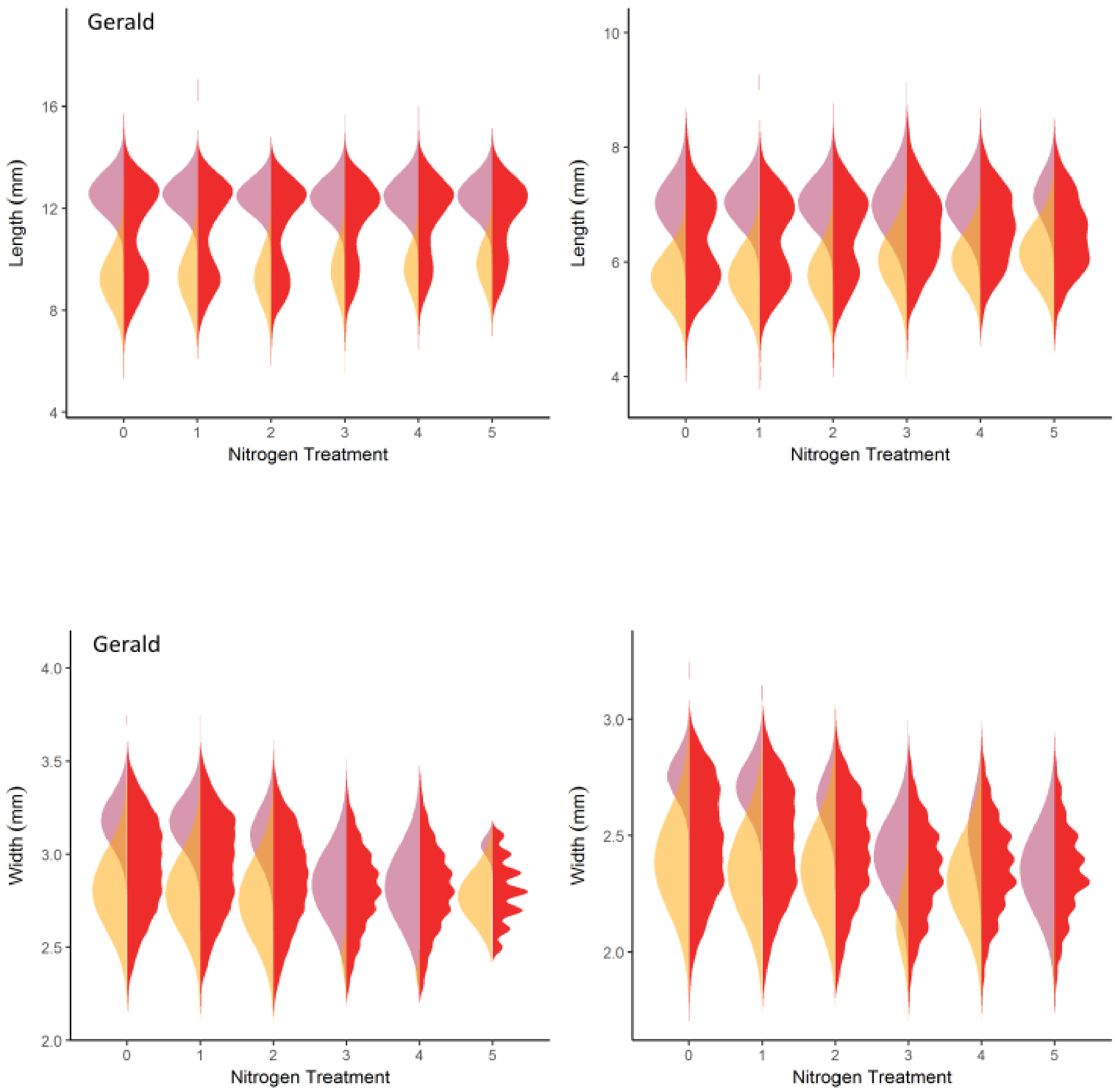


Figure 4.2*. *Frequency of individual grain and groat length and width of Mascani, at ADAS 2015 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

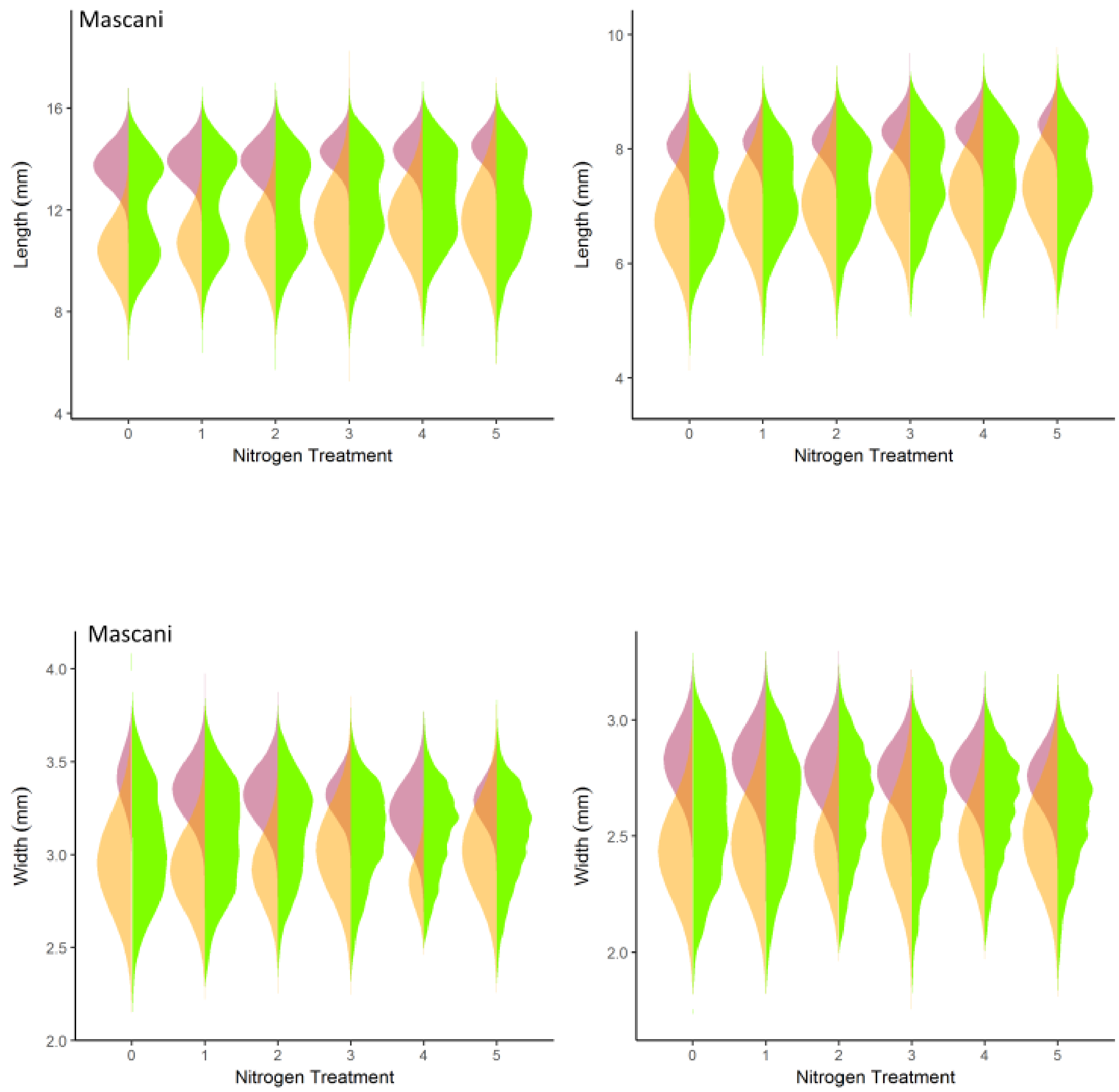


Figure 4.2*. *Frequency of individual grain and groat length and width of Tardis, at ADAS 2015 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side*

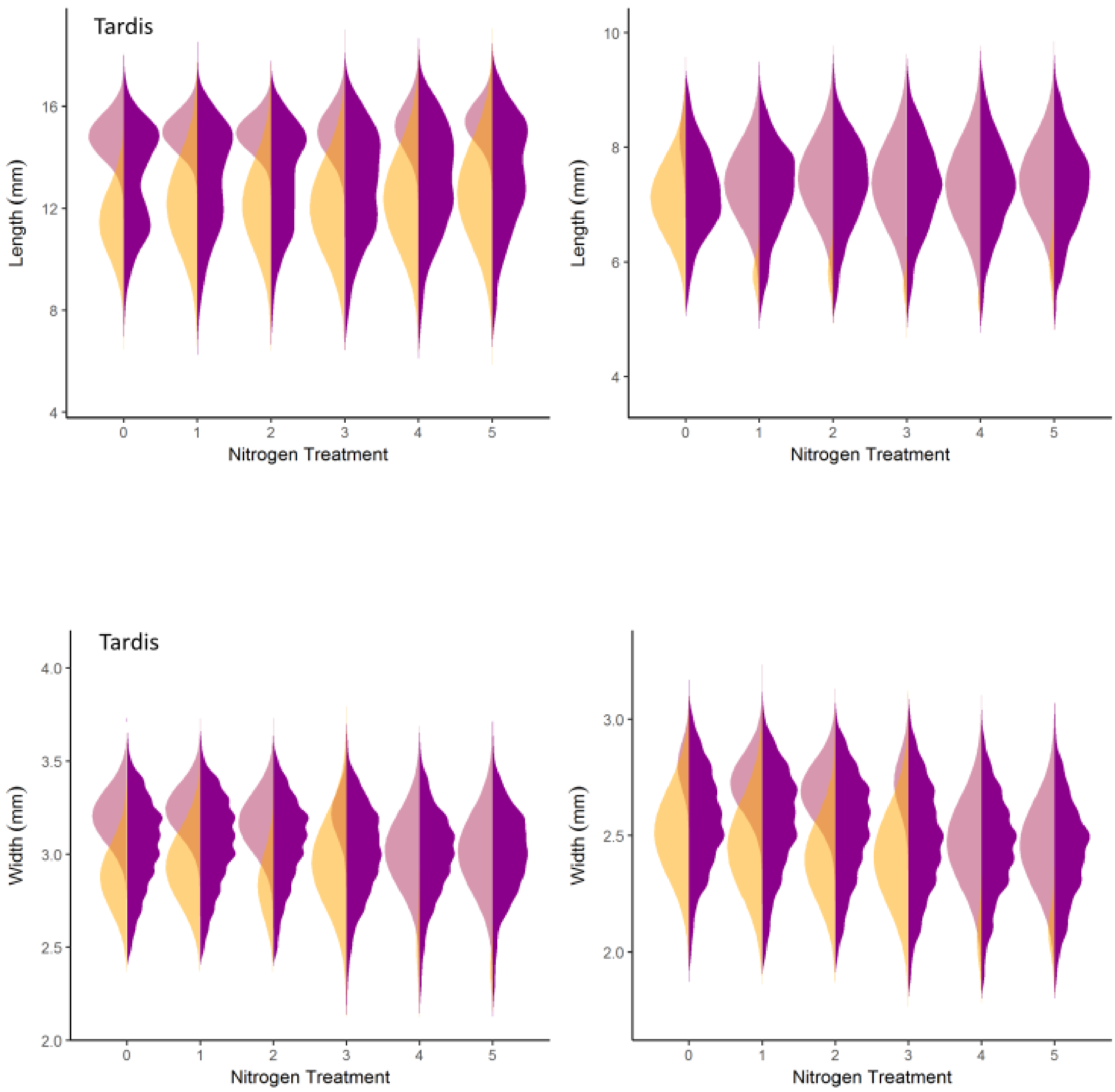


Figure 4.2*. *Frequency of individual grain and groat area, length and width of Balado, at ADAS 2014 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

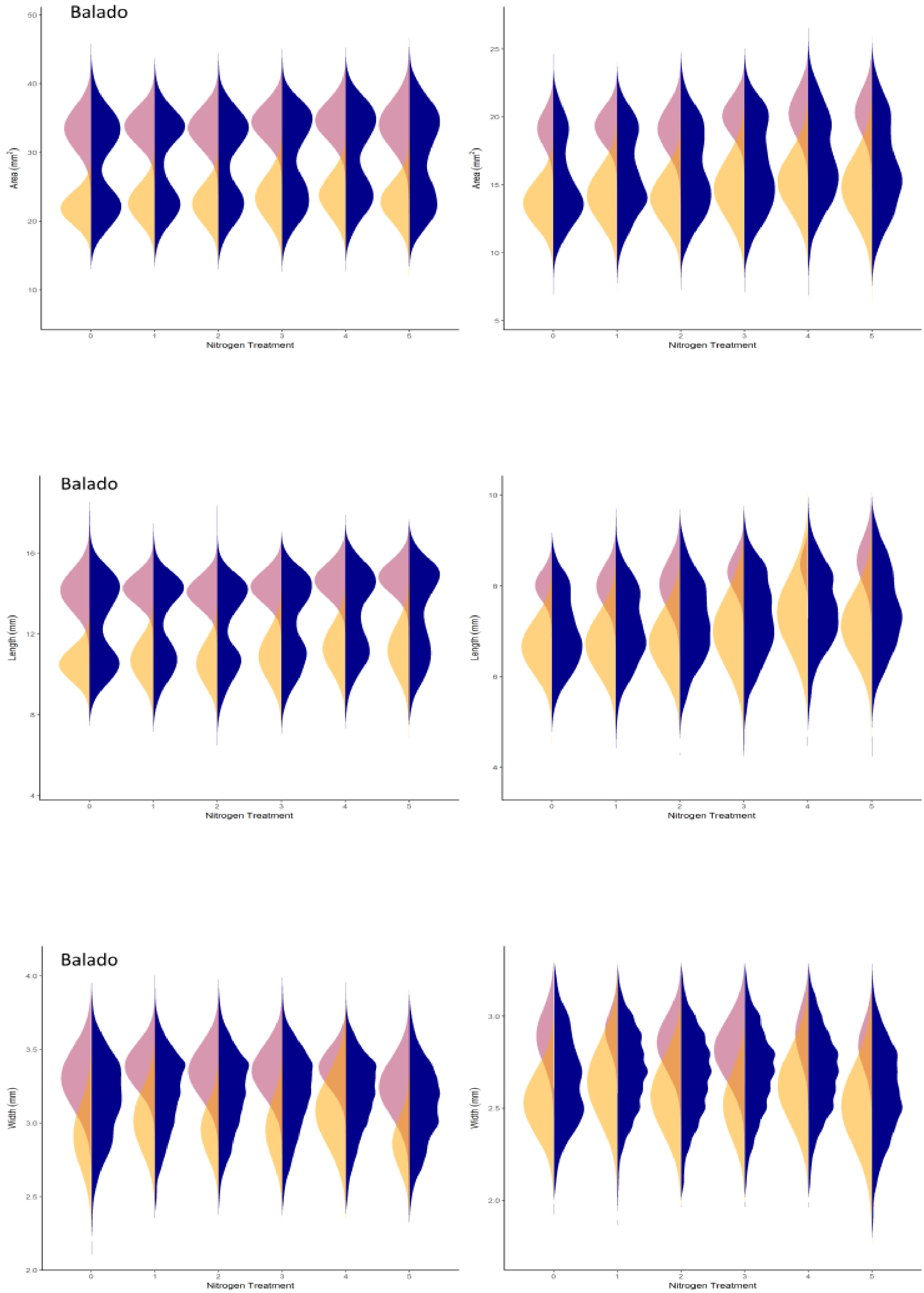


Figure 4.2*. *Frequency of individual grain and groat area, length and width of Balado, at IBERS 2014 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

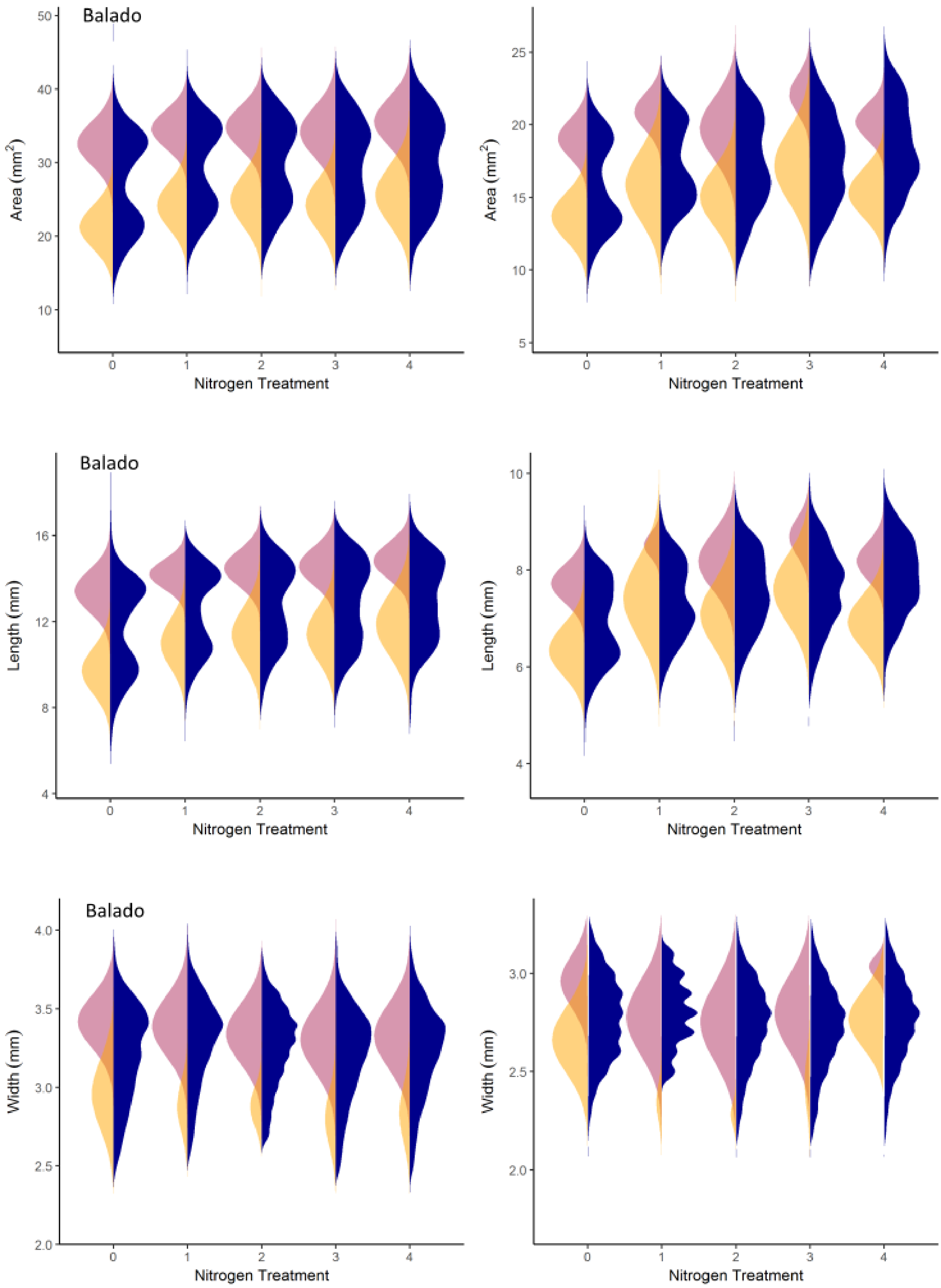


Figure 4.2*. *Frequency of individual grain and groat area, length and width of Gerald, at ADAS 2014 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

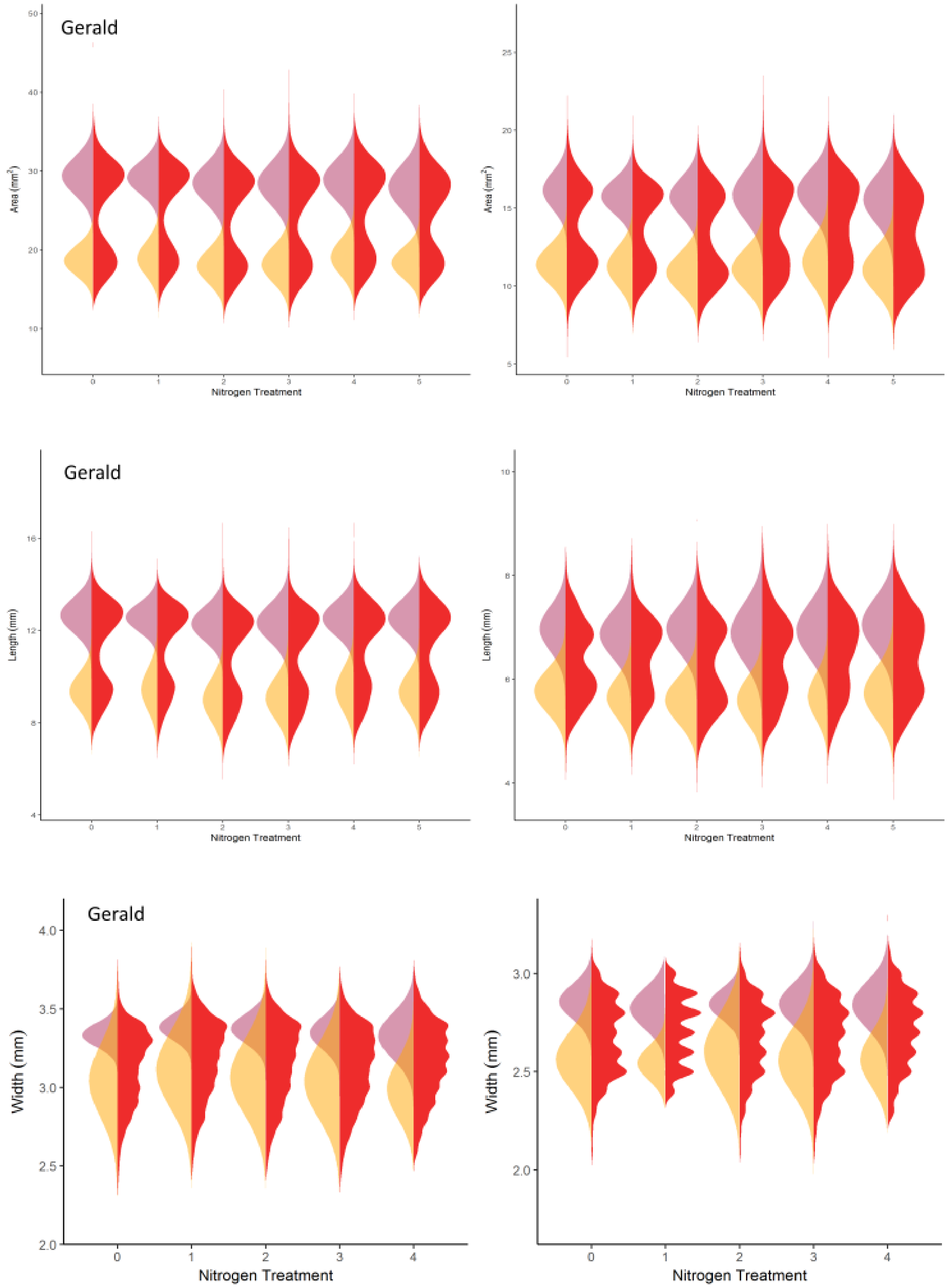


Figure 4.2*. *Frequency of individual grain and groat area, length and width of Gerald, at IBERS 2014 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

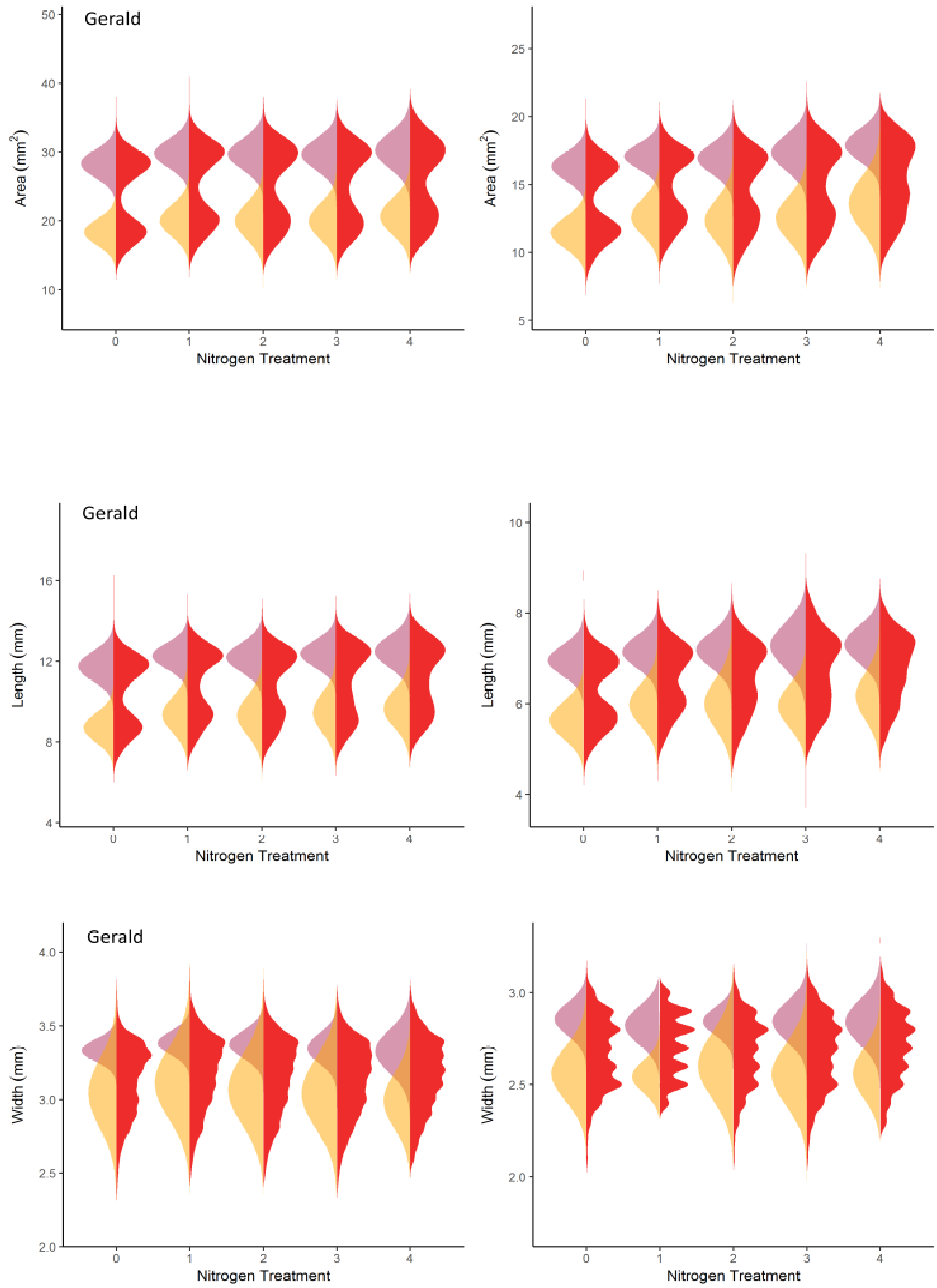


Figure 4.2*. *Frequency of individual grain and groat area, length and width of Mascani, at ADAS 2014 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

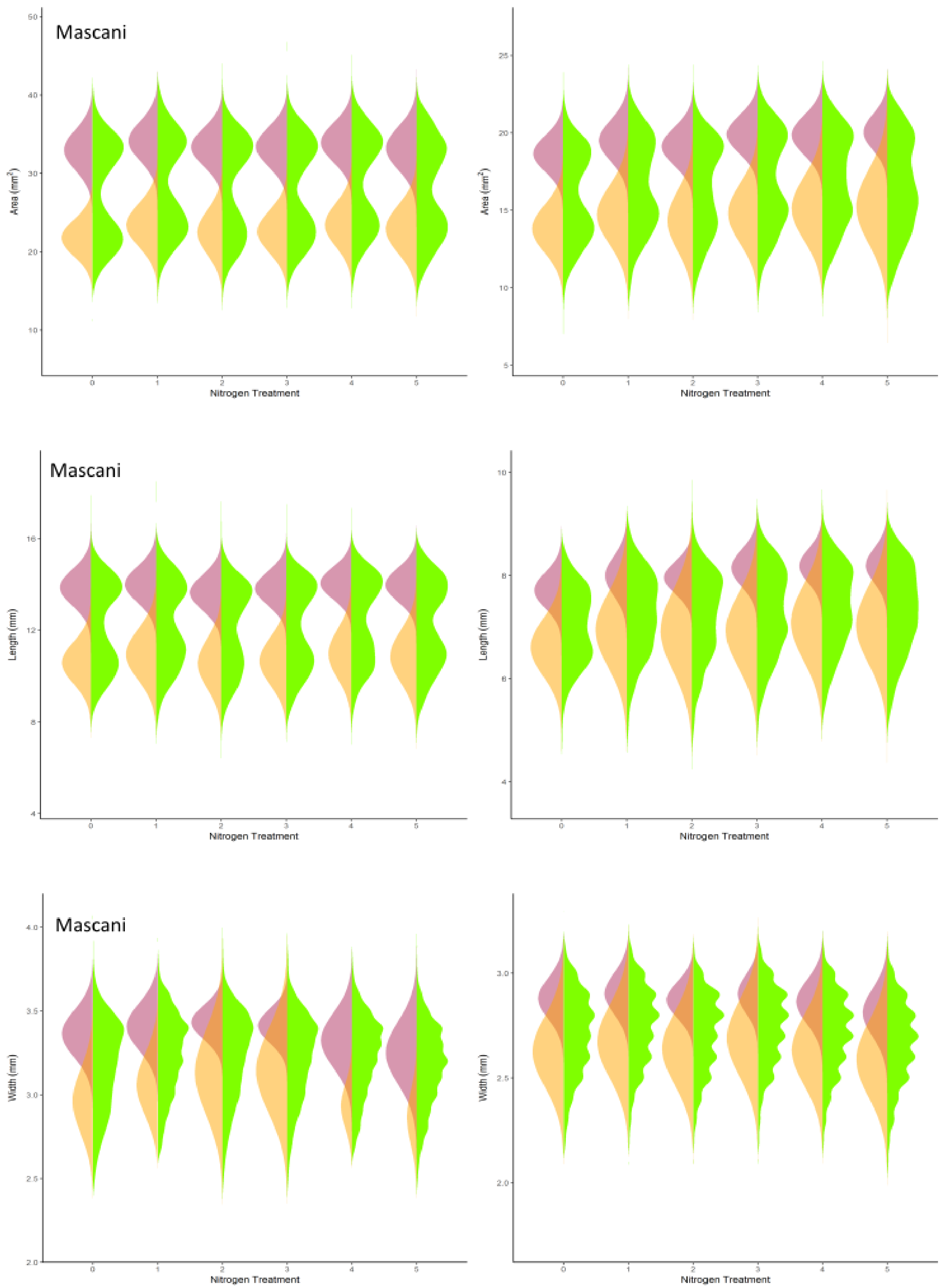


Figure 4.2*. *Frequency of individual grain and groat area, length and width of Mascani, at IBERS 2014 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

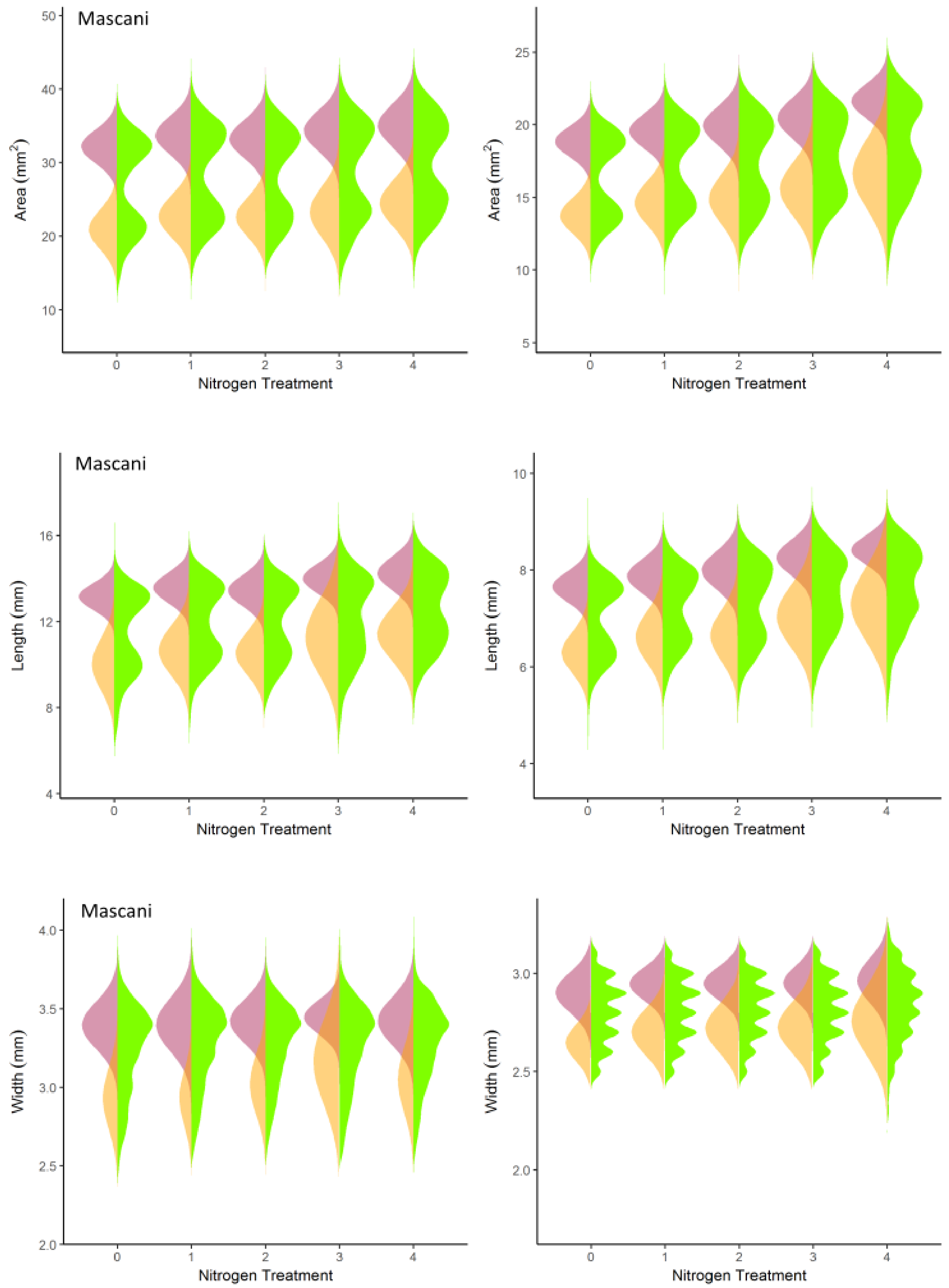


__Figure 4.2.__ Frequency of individual grain and groat area, length and width of Tardis, at ADAS 2014 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

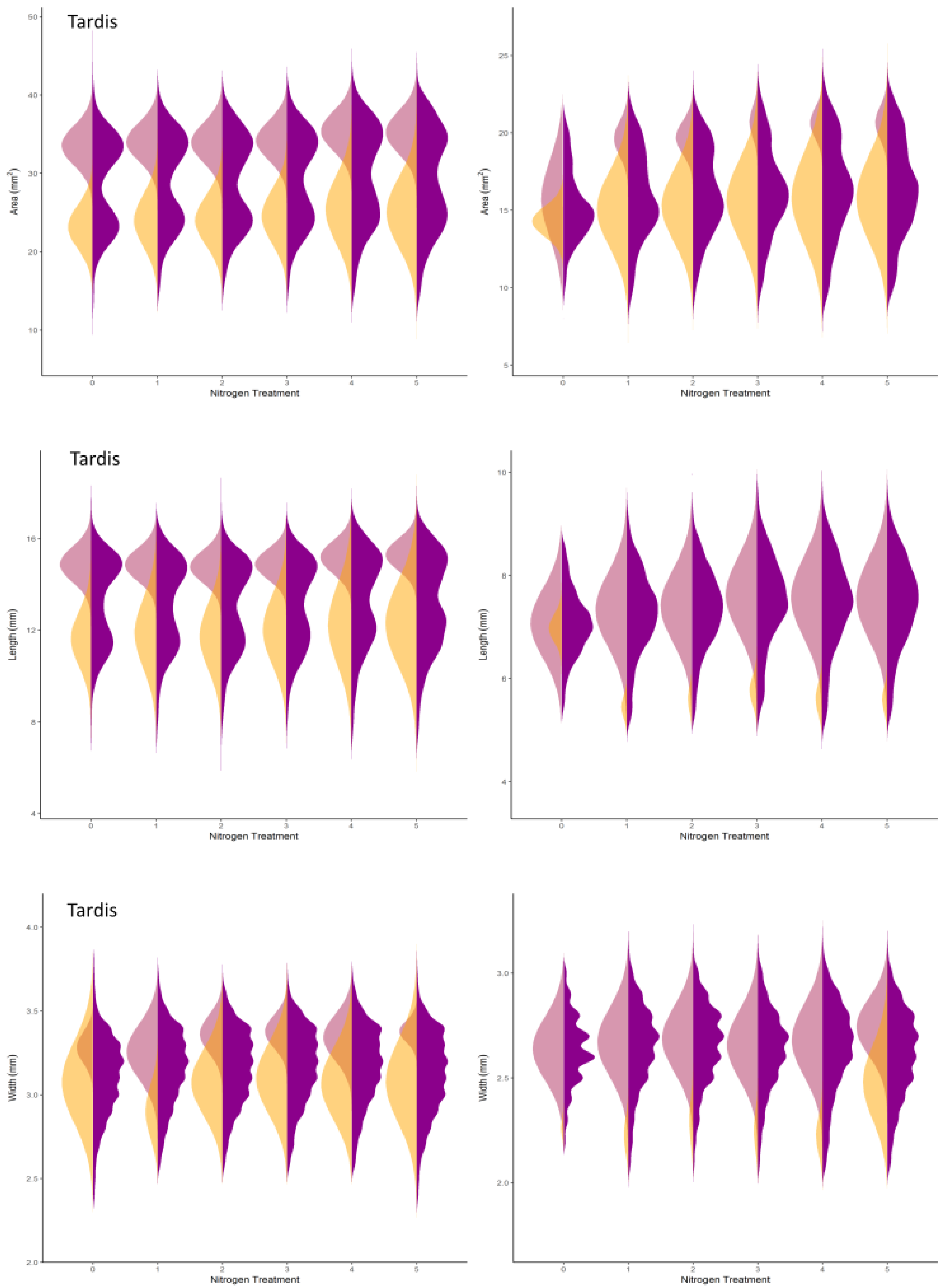
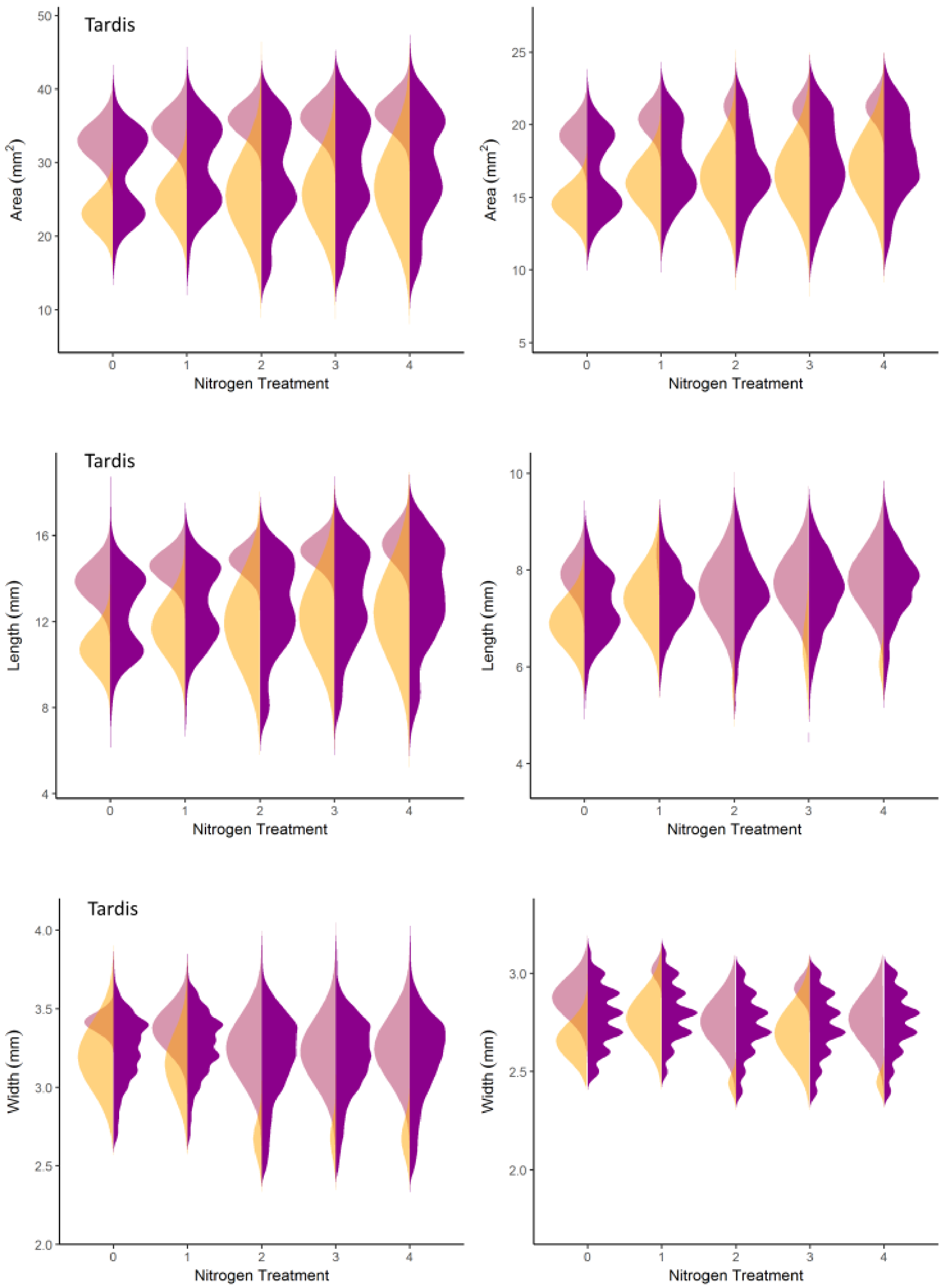
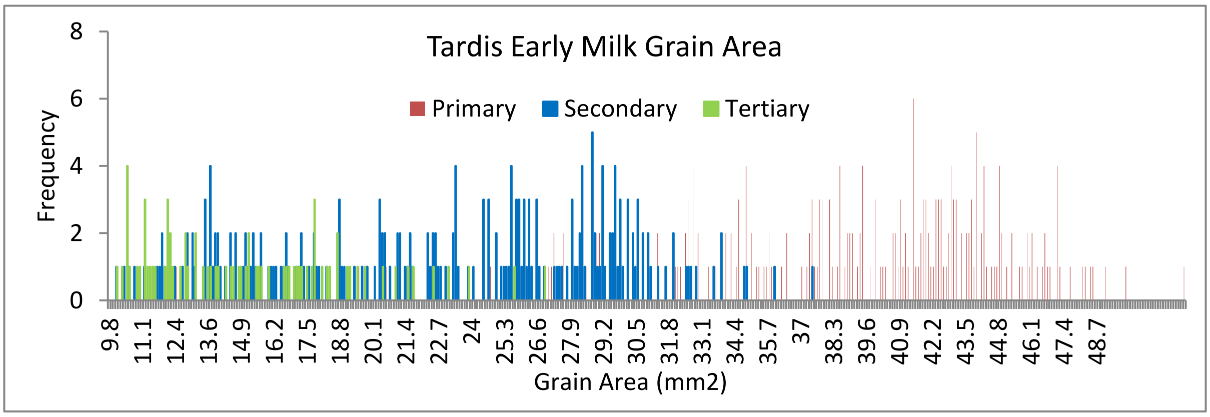


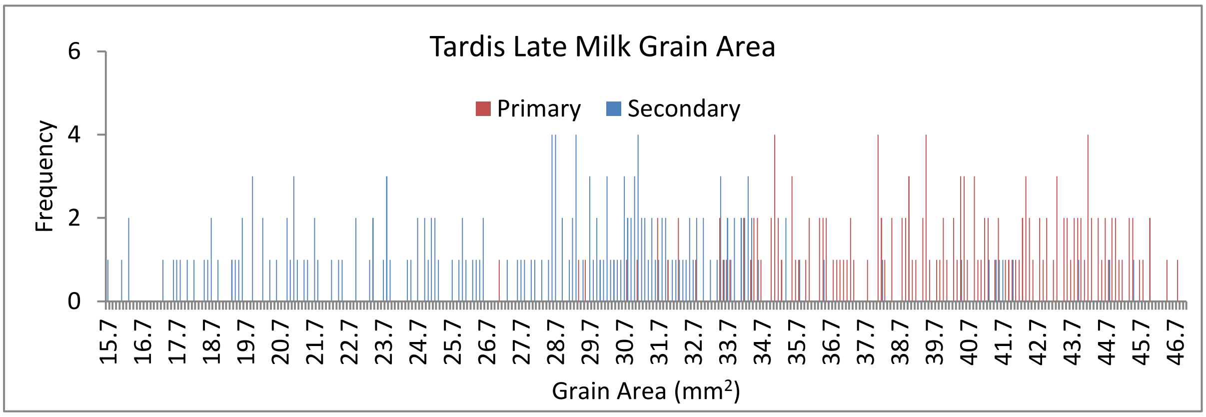
Figure 4.2*. *Frequency of individual grain and groat area, length and width of Tardis, at IBERS 2014 with increasing levels of nitrogen. A frequency plot is shown on the right-hand side area for each nitrogen level and the fitted bimodal distribution is shown on the left-hand side.*

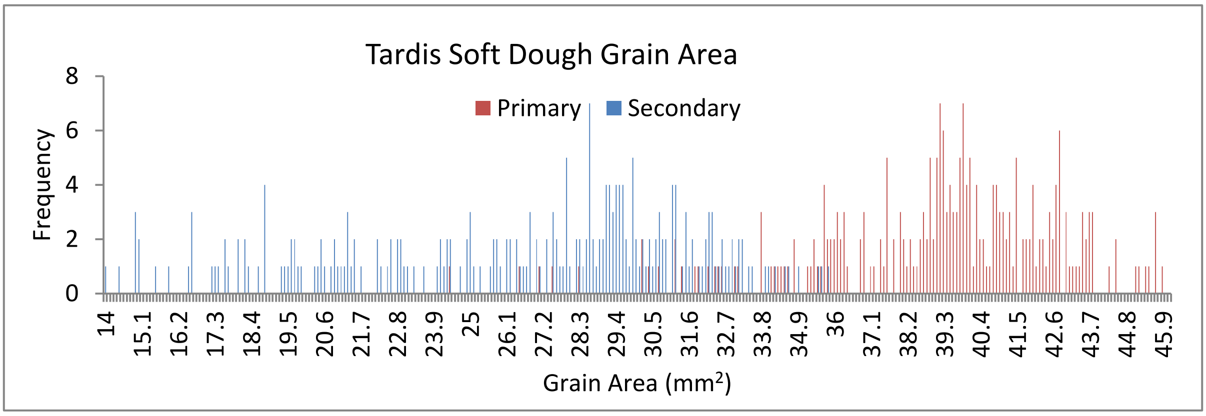


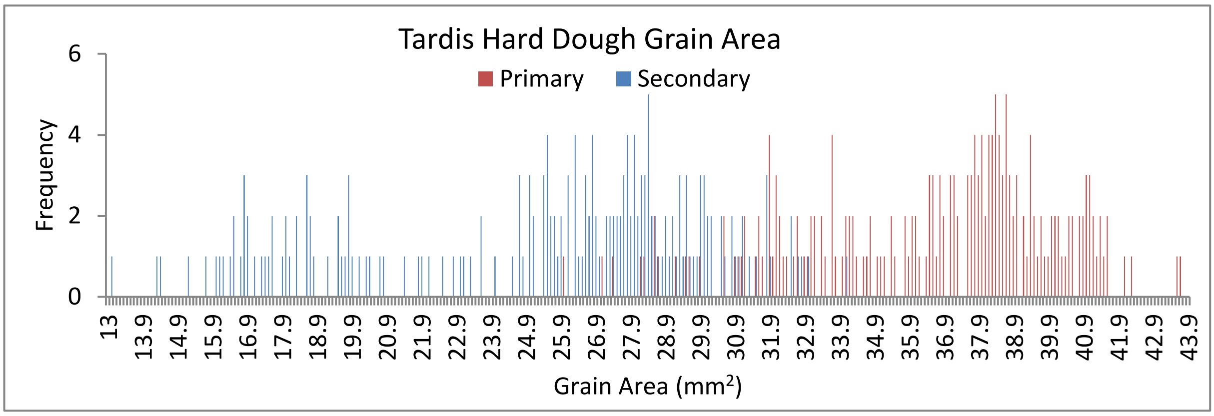
5.3.5.1 Grain size

Figures 5.4* *Frequency grain area (mm^2) histograms along growth development stages of Tardis primary and secondary grain.*

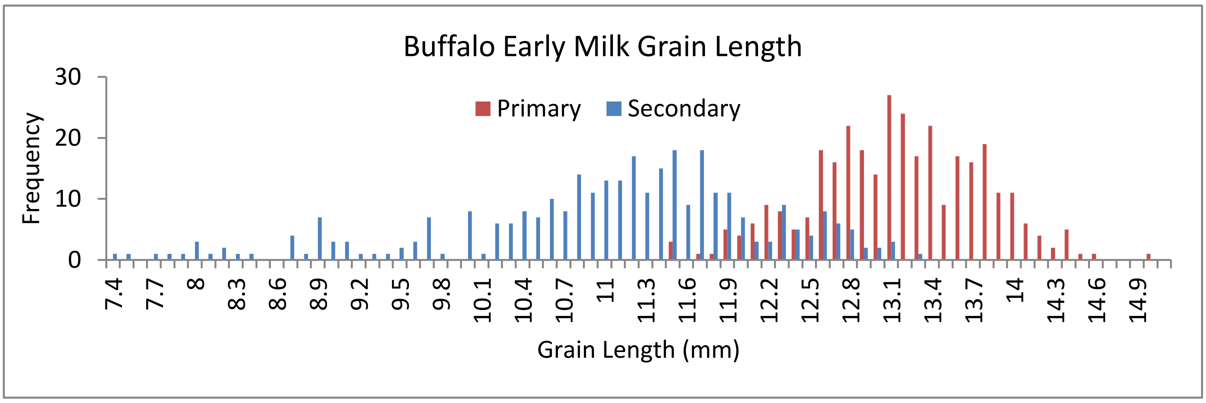


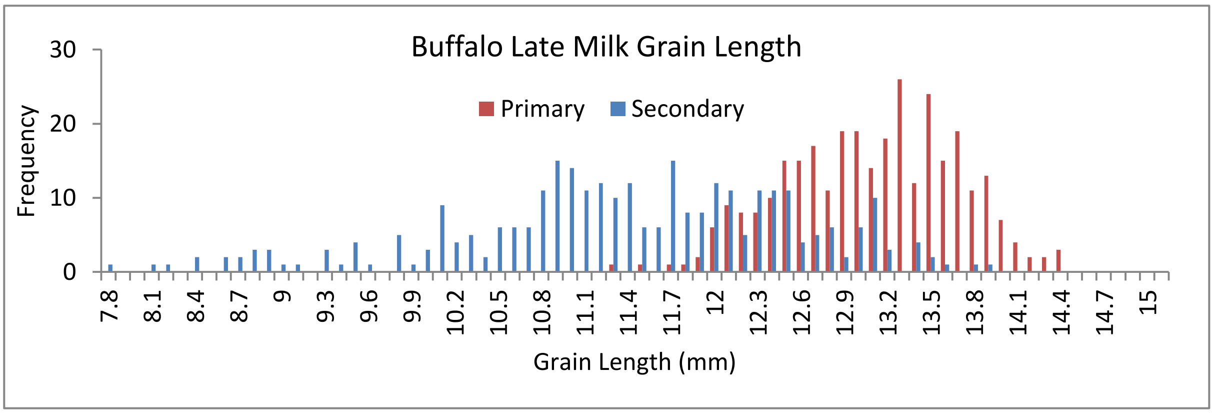


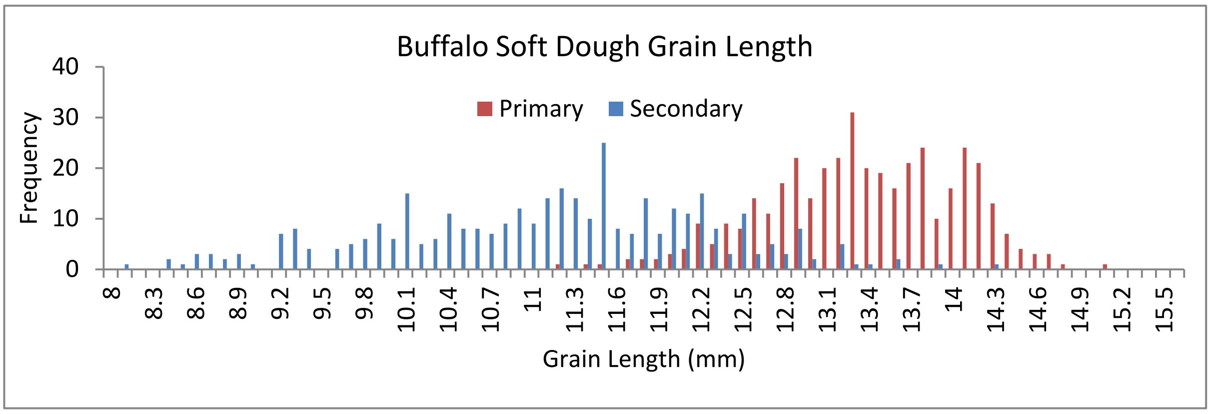


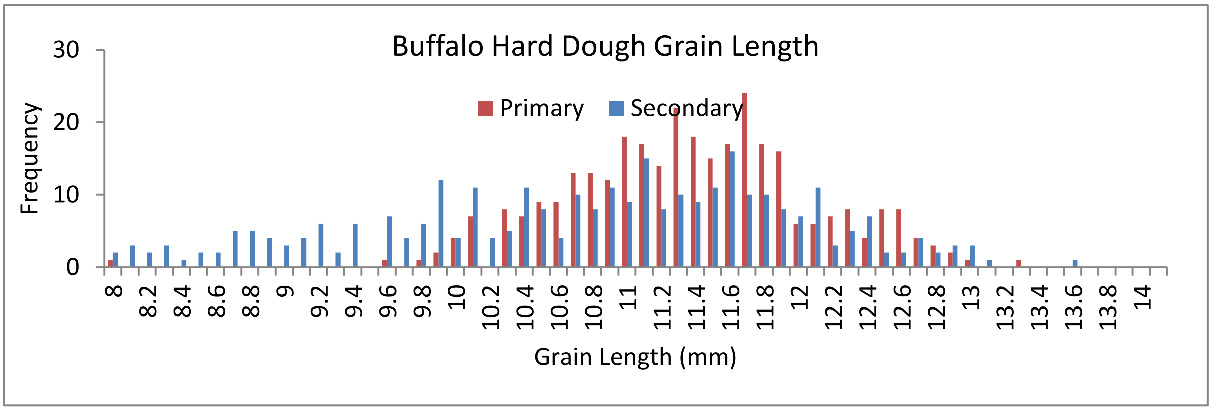


Figures 5.4* *Frequency grain length (mm) histograms along growth development stages of Buffalo primary and secondary grain.*

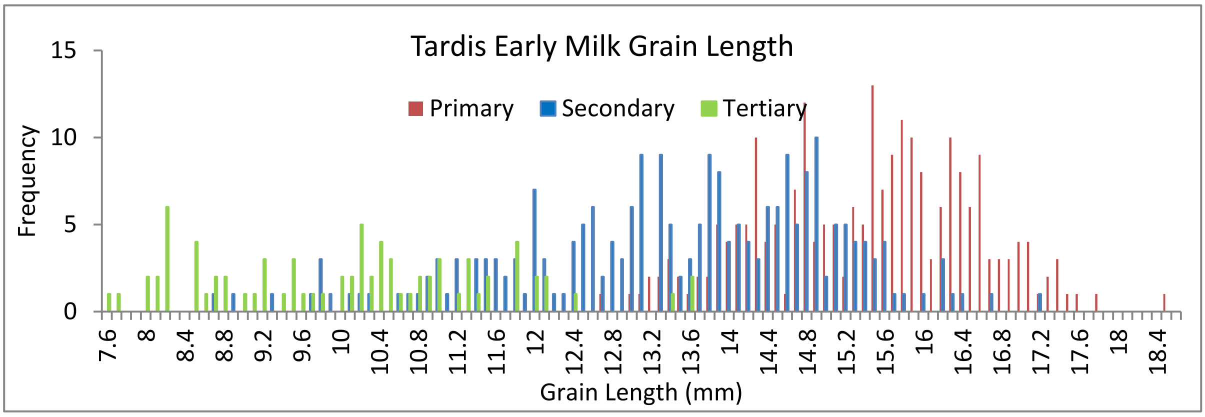


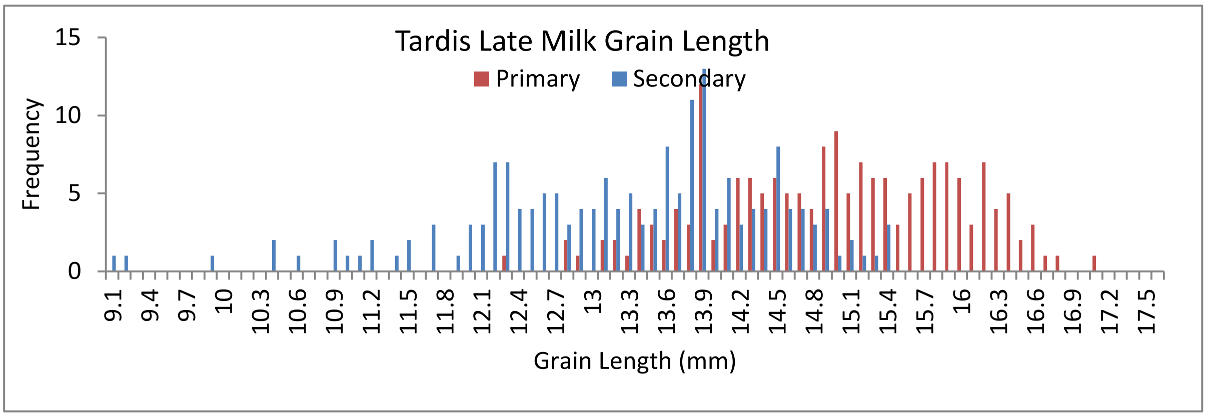


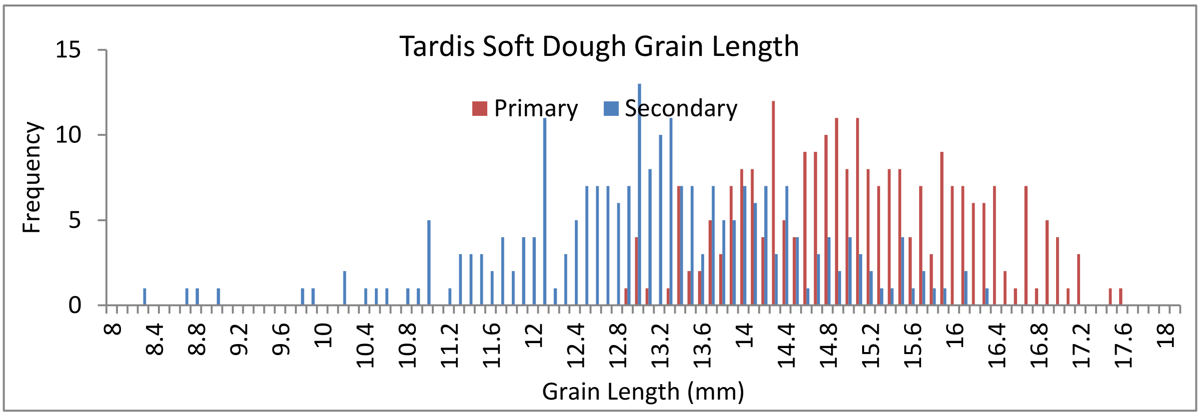


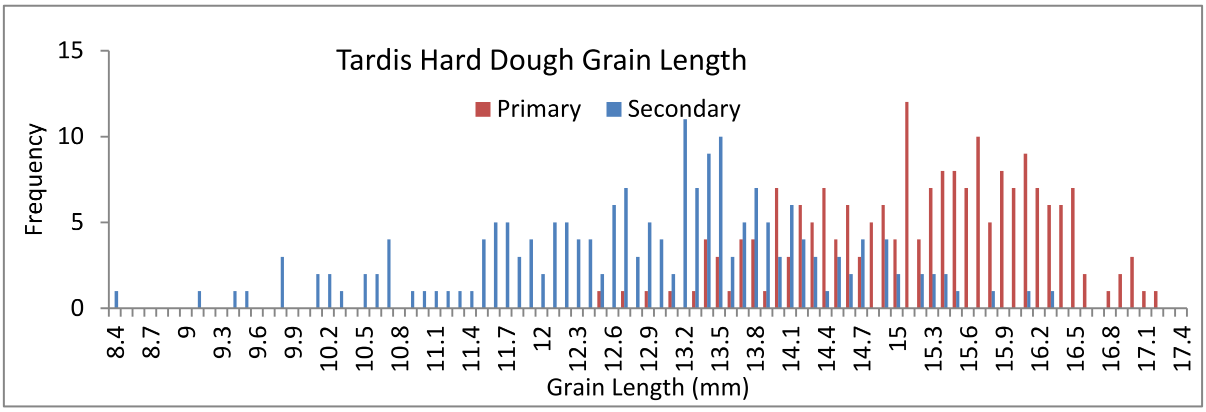


Figures 5.4* *Frequency grain length (mm) histograms along growth development stages of Tardis primary and secondary grain.*

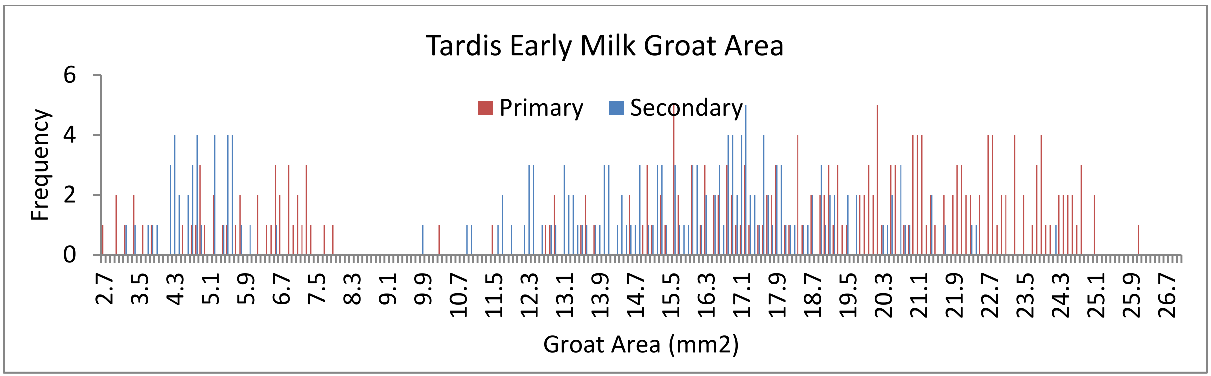


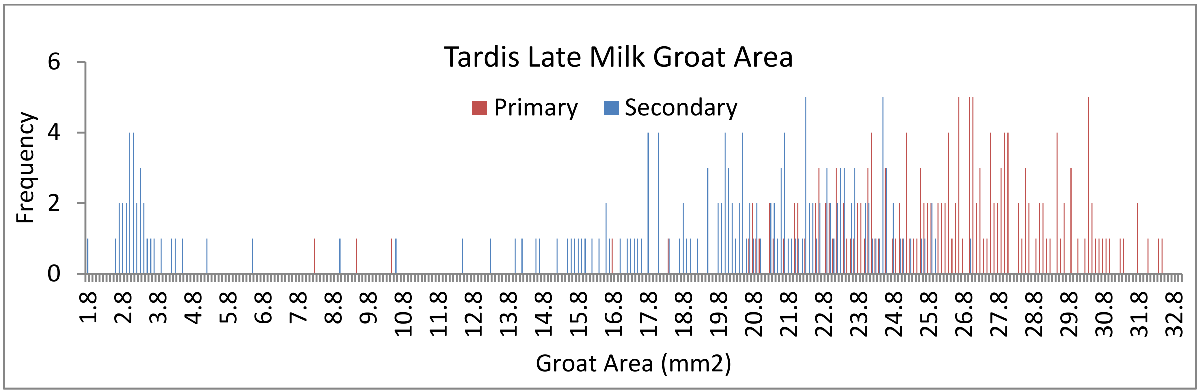

Tardis Late Milk Grain Length
Primary
Secondary
Frequency
Grain Length (mm)

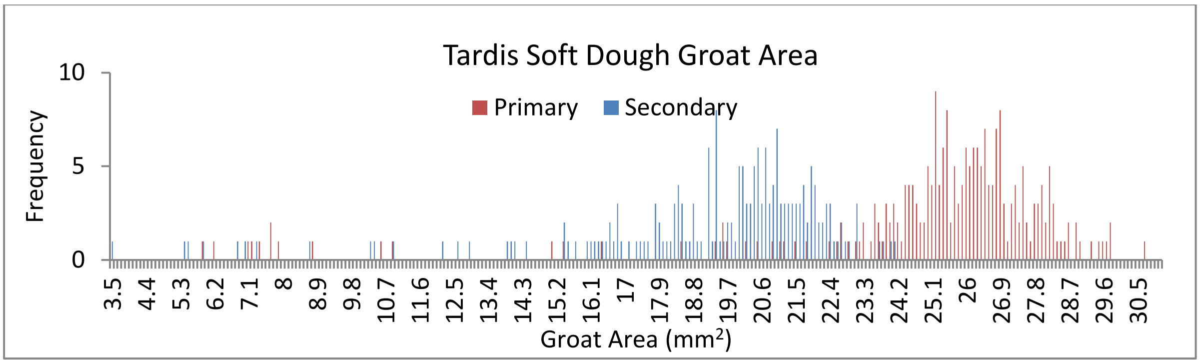

Tardis Soft Dough Grain Length
Primary
Secondary
Frequency
Grain Length (mm)

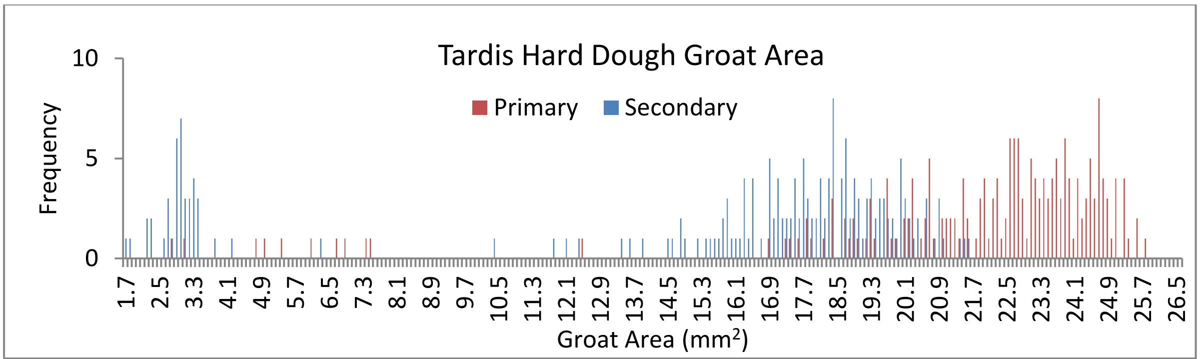

Tardis Hard Dough Grain Length
Primary
Secondary
Frequency
Grain Length (mm)

*__Figures 5.4*__ Frequency groat area (mm^2) histograms along growth development stages of Tardis primary and secondary grain.*

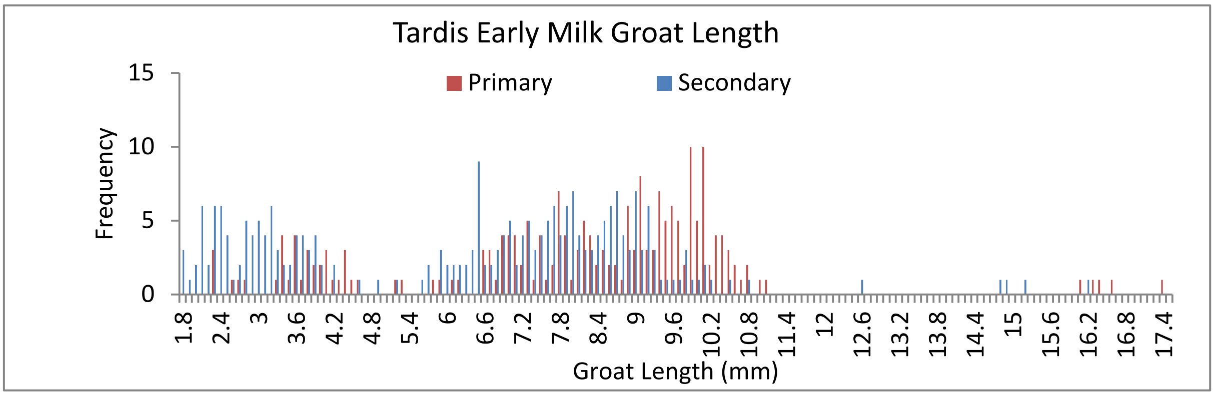


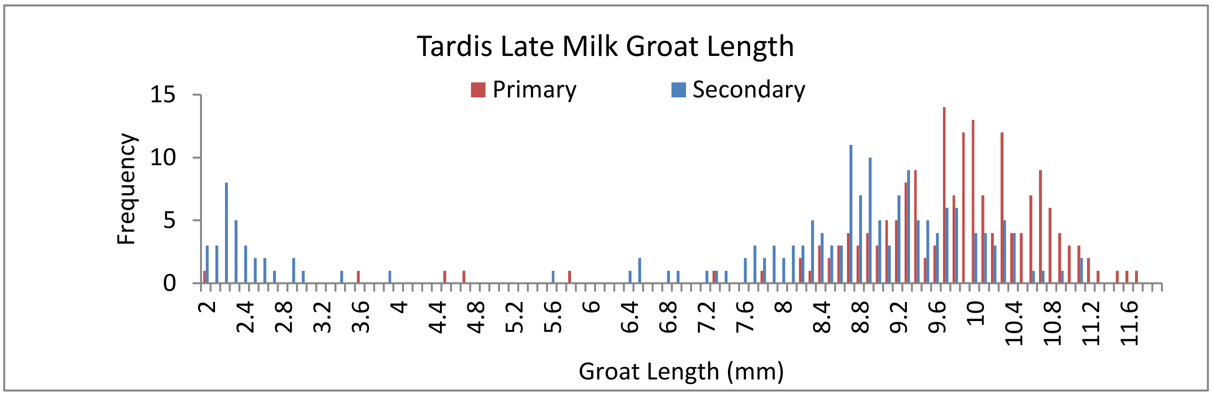


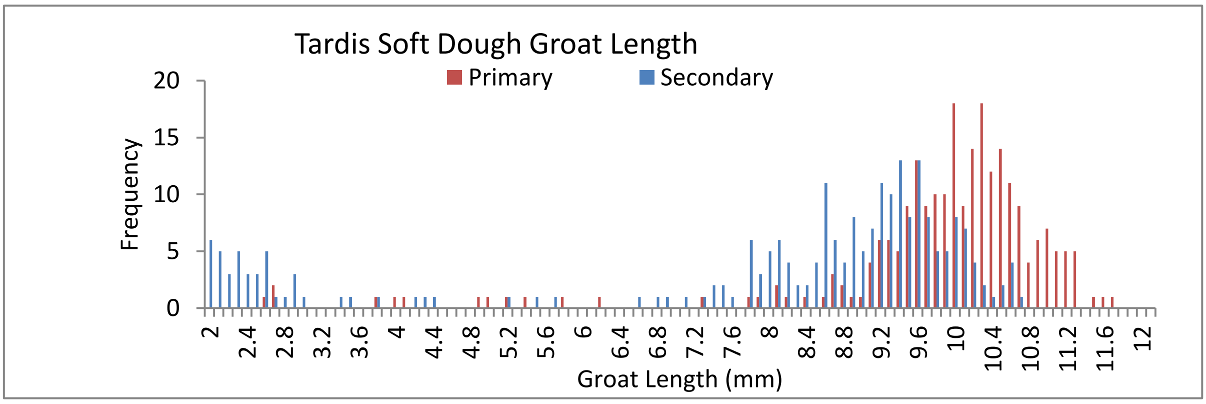


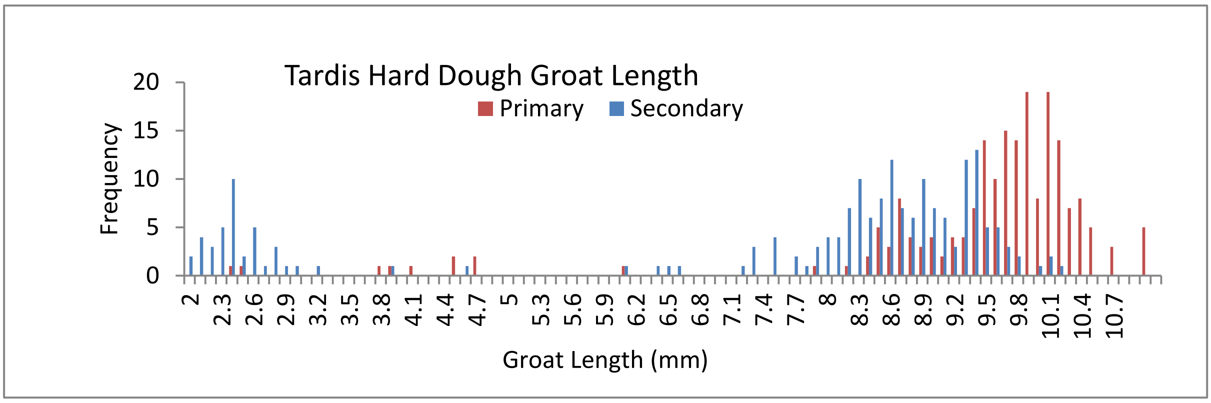


Figures 5.4* *Frequency groat length (mm) histograms along growth development stages of Tardis primary and secondary grain.*

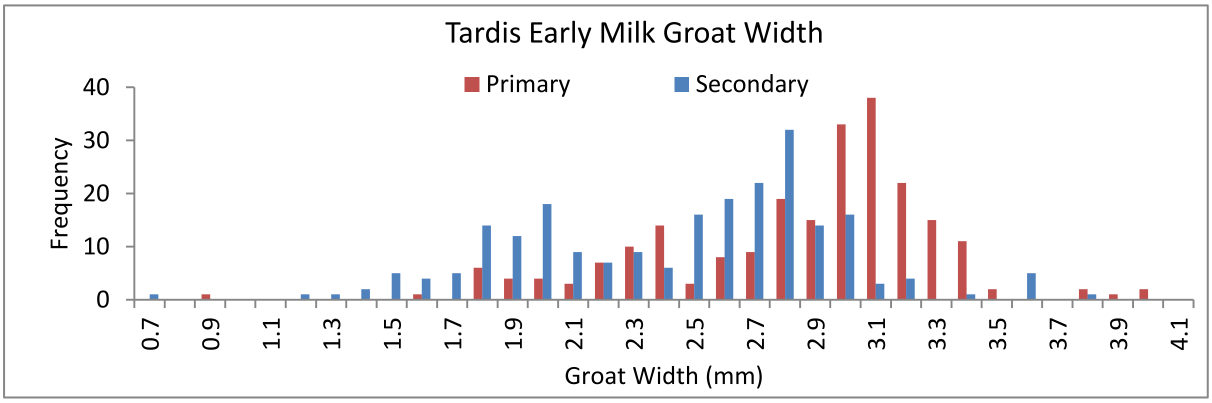


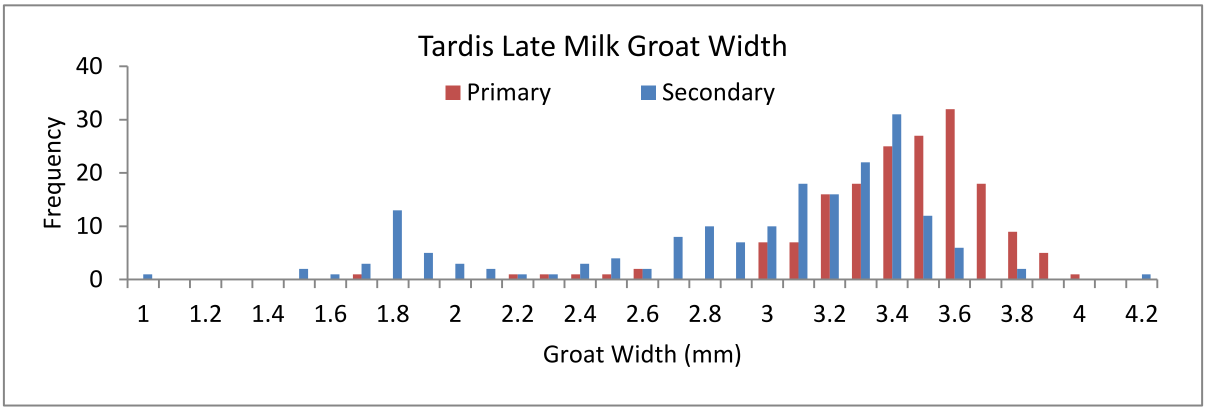


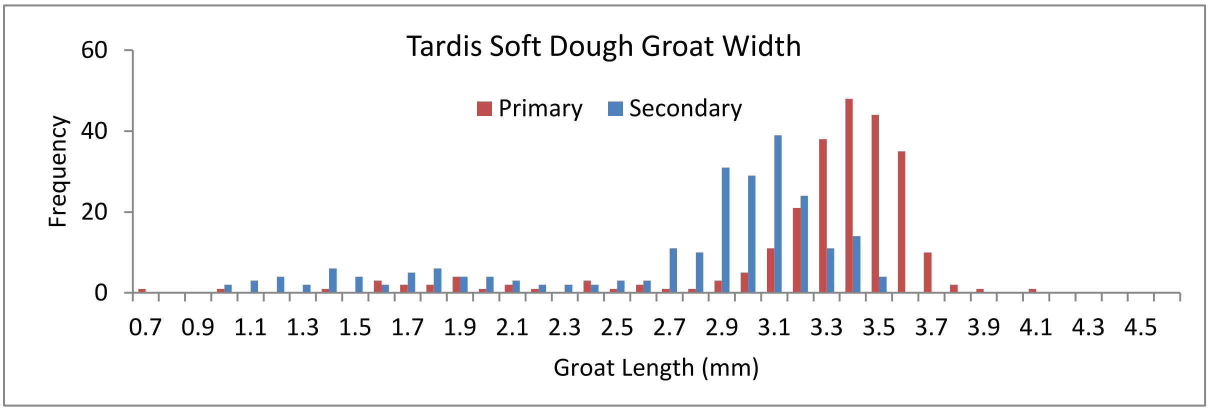


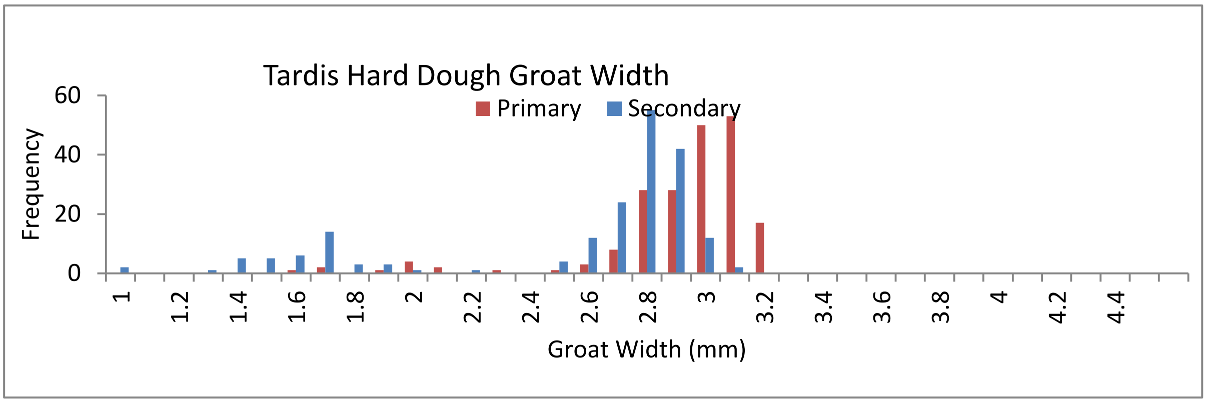


Figures 5.4* *Frequency groat width (mm) histograms along growth development stages of Tardis primary and secondary grain.*






Tardis Soft Dough Groat Width
Primary
Secondary
Frequency
60
40
20
0
0.7
0.9
1.1
1.3
1.5
1.7
1.9
2.1
2.3
2.5
2.7
2.9
3.1
3.3
3.5
3.7
3.9
4.1
4.3
4.5
Groat Length (mm)


Tardis Hard Dough Groat Width
Primary
Secondary
Frequency
60
40
20
0
1
1.2
1.4
1.6
1.8
2
2.2
2.4
2.6
2.8
3
3.2
3.4
3.6
3.8
4
4.2
4.4
Groat Width (mm)